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The relationship between protein and phosphorus digestion and retention in growing pigs and broiler chickens

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THE RELATIONSHIP BETWEEN PROTEIN AND PHOSPHORUS DIGESTION AND RETENTION IN GROWING PIGS AND BROILER CHICKENS

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Dedicated to my dear family

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LIST OF ABBREVIATIONS

SYMBOL	DESCRIPTION
AA	Amino acid
ADF	Acid detergent fiber
AIA	Acid-insoluble ash
AID	Apparent ileal digestibility
ATTD	Apparent total tract digestibility
ATTDP	Apparent total tract digestible phosphorus
ATTR	Apparent total tract retention
BEL	Basal endogenous losses
BW	Body weight
Ca	Calcium
CM	Canola Meal
CP	Crude protein
DDGS	Distillers’ dried grains with solubles
DM	Dry mater
EPL	Endogenous P losses
IAA_{end}	Ileal endogenous losses of AA
MCP	Mono-calcium phosphate

NaPiII-b	Sodium-dependent phosphate transporter II-b
Nitrogen	N
NDF	Neutral detergent fiber
NFD	Nitrogen-free diet
P	Phosphorus
SBM	Soybean meal
SEM	Standard error of the means
SID	Standardized ileal digestibility
STTD	Standardized total tract digestibility
TID	True ileal digestibility
TTTD	True total tract digestibility
TTTR	True total tract retention

ABSTRACT

Xue, Pengcheng. Ph.D., Purdue University, December 2016. The Relationship between Protein and Phosphorus Digestion and Retention in Growing Pigs and Broiler Chickens. Major Professor: Dr. Layi Adeola.

The objective of this study was to investigate the relationship between protein and P digestion and retention in growing pigs and broiler chickens. The methodology of determining the digestibility of AA and P and the effect of dietary N and P on the digestion and retention of these two nutrients were investigated.

An experiment was conducted in growing pigs to investigate the additivity of AID or SID of CP and AA in mixed diets containing multiple protein sources. Using the determined AID or SID for CP and AA in corn, SBM, DDGS, and canola meal, the AID or SID for 4 mixed diets was predicted and compared with determined AID or SID, respectively. Eighteen growing pigs (initial BW = 61.3 ± 5.5 kg) with surgically fitted T-cannula were assigned to a duplicated 9 × 4 incomplete Latin square design with 9 diets and 4 periods. A nitrogen-free diet was included to estimate basal ileal endogenous loss of AA; 4 semi-purified diets to determine the AID and SID of CP and AA in the 4 ingredients; and 4 mixed diets to test the additivity of AID and SID. The results substantiate the notion that SID of AA are more accurate than AID for predicting ileal digestibility of AA in mixed diets containing multiple protein sources.

To determine the TTTD of P in triticale DDGS for growing pigs with or without phytase using the regression method, six diets were formulated in a 3 × 2 factorial arrangement, including 3 levels of triticale DDGS (300, 400, or 500 g/kg) and phytase (0 or 500 FTU/kg of diet). A total of 48 barrows (initial BW 22.2 ± 1.3 kg) were assigned to the 6 diets in a randomized complete block design. There was a 5-d adjustment period followed by a 5-d total collection of feces. The results indicated that phytase improved ATTD of P in triticale DDGS ($P < 0.001$). In diets without added phytase, the ATTD of P in triticale DDGS was 65.0, 67.7, and 63.2% for the diets with 300, 400, and 500 g/kg triticale DDGS, respectively; the corresponding values for diets with added phytase were 77.3, 76.3, and 75.7%.The TTTD of P was estimated at 75.4% for triticale DDGS or 81.1% with added phytase, respectively. The difference between the TTTD with or without phytase was not statistically significant. For triticale DDGS, the supplementation of 500 FTU/kg phytase in the diet could increase the ATTD of P ($P < 0.001$), but not the TTTD of P.

The effect of dietary CP concentrations on ileal P digestion in growing pigs was investigated in the third study. A total of 18 ileal-cannulated pigs (initial BW 44.2 ± 3.2 kg) were used in a duplicated 9 × 3 incomplete Latin Square design, with 9 treatments and three 7-d experimental periods giving 6 replicates per treatment. The 9 treatments consisted of 1 nitrogen-free diet to estimate basal endogenous loss of AA, and 8 corn-soybean meal-based diets in a 2 × 4 factorial arrangement, which included 2 CP concentrations (6.9 or 13.4%) and 4 ATTDP concentrations (0.09, 0.16, 0.24, or 0.32%). Low CP diets limited ileal digested P (g/kg•DMI) (P < 0.05). The ileal digested P (g/kg•DMI) increased linearly ($P < 0.01$) with increasing ATTDP concentrations in the low CP group, but the pattern was linear (*P* < 0.01) and quadratic (*P* < 0.01) in the high CP group. In the low and high CP diets, the determined true ileal digestibility of P in mono-calcium phosphate was 54.4% and 75.6%, respectively. In conclusion, this research indicated that the ileal digestion of P could be limited by protein deficiency. Thus dietary CP concentration should be considered in P digestibility related studies.

The quantitative relationship between N and P digestion and retention in broiler chickens and growing pigs was determined in the last two studies. In the broiler chicken study, a total of 384 14-d-old male broiler chickens were used in a randomized complete block design with 8 treatments and 6 replicates per treatment in a 7-d experimental period. There were 8 corn-soybean meal-based diets in a 2 × 4 factorial arrangement, which included 2 CP concentrations (10.7 or 21.5%) and 4 ATTDP concentrations (0.18, 0.32, 0.45, or 0.59%). Results showed that low dietary CP concentration limited growth performance (*P* < 0.01), pre-cecal digestion and total tract retention of P (*P* < 0.01), and NaPi-IIb gene expression (*P* < 0.05). Pre-cecal digestion and total tract retention of P (g/kg DM intake) linearly increased (*P* < 0.01) with increasing ATTDP concentrations in both low and high CP groups. In conclusion, this study suggests an interrelationship between N and P digestion such that CP deficiency decreased the growth performance of birds consequently reducing pre-cecal P digestion in broiler chickens. Total tract retention of CP and P are linked with each other and body tissue growth may be a driver of the deposition of these two nutrients. In the growing pig study, a total of 72 growing pigs (initial BW 20.9 ± 0.8 kg) were used in a randomized complete blocked design, with 9 treatments and four 10-d experimental periods giving 8 replicates per treatment. The pigs were blocked by BW and allotted to 9 treatments with a 3 × 3 factorial arrangement consisting of 3 CP concentrations (5.5, 9.7, or 13.9%) and 3 ATTDP concentrations (0.11, 0.19, or 0.27%). The determined TTTD of P in MCP for the 5.5, 9.7, and 13.9% CP diets were 80.5, 82.6, and 87.9%, respectively. There were no statistical differences among the three TTTD estimates. In the nitrogen utilization results, increasing dietary P level decreased the urine nitrogen output ($P < 0.05$). In conclusion, the results indicated that dietary CP deficiency may limit total tract P digestion and retention. Yet the quantitative relationship between total tract N and P retention remains unclear.

In summary, the SID of most AA in corn, SBM, canola meal, and DDGS, are additive in complete diets. The regression method can be used to determine the true P digestibility and retention in feed ingredients for growing pigs and broiler chickens. Dietary CP deficiency can limit ileal and total tract P digestion. In broiler chickens, the retention of P is correlated with the retention of N. Yet in growing pigs this relationship has not been observed. Body weight gain might be the driving factor for the correlation between N and P retention in growing pigs and broiler chickens.

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

In the accretion of lean tissue of animals, dietary supplementation of amino acids (AA) and phosphorus (P) plays critical roles in their performance and efficiency. As fundamental building blocks for protein, AA supplementation is the key for protein synthesis in the animal body (Young, 1976). Phosphorus is a macro mineral element widely distributed in the animal body. The nutrition of P is of great importance to the growth of skeletal structure of animals (Rowland et al., 1967). Besides bone health, P also plays roles in cell membrane formation and many aspects of cell metabolism such as regulating biosynthesis and signaling pathways. The nutrition of these two nutrients can have great impact on the weight gain and feed efficiency of animals (Gómez et al., 2002; Zhai and Adeola, 2013). In animal production industry, one of the main purposes is meat production for human consumption. Thus the nutrition of AA and P is important for growing pigs and broiler chickens. In feed for animals, the components that provide protein, or AA, and P to the animal account for a large portion of the cost of feed. Meanwhile, excessive excretion of N and P from animals to the environment may lead to several issues such as eutrophication in the water system. For these reasons, accurate dietary supplementation of AA and P is critical for optimal performance of the animal, as well as the economic and environmental pressures from the animal production industry.

To determine the digestibility of AA or P in feed ingredient, the principle can be expressed by the following equation:

$$\text{Digestibility, \%} = 100 \times (\text{Nutrient}_{I} - \text{Nutrient}_{O}) / \text{Nutrient}_{I},$$

where the Nutrient_{I} and Nutrient_{O} stand for the intake and output quantity of the nutrient, respectively. This equation can be applied to calculate the digestibility of nutrients in feed ingredients if the total amount of nutrient intake and output are measured during the experiment. Nutrient intake can be calculated based on feed allowance and analyzed nutrient composition. The nutrient output, on the other hand, can be measured on total tract or ileal digesta basis, depending on the focus of the study and the nutrient that is investigated.

1.2 Amino acids

The evaluation of AA in feed ingredients should be performed on ileal digestion basis, because of the limited ability of AA absorption and the impact of microbial fermentation in the hind gut (Laplace et al., 1985).The ileal AA digestibility of AA in feed ingredients for poultry and swine has been an important research topic in the last few decades (Stein et al., 2007). Among the methods for collecting ileal digesta, collection from the ileum after animals are euthanized is the most common method in poultry, and through surgically fitted simple T-cannula in pigs (Kadim and Moughan, 1997; Stein et al., 2007). There are other methods for collecting ileal digesta from animals, such as through the use of cecectomized roosters and ileocecal anastomosis in pigs, which are well documented in previous studies and reviews (Payne et al., 1971; Sauer et al., 2001).

The basic equation for apparent ileal digestibility (AID) estimation is:

$$\text{AID, \%} = [(\text{AA intake} - \text{ileal AA output})/\text{AA intake}] \times 100.$$

However, for some of the ileal digesta collection methods mentioned above, the quantity of ileal AA output has to be estimated by an index marker, because in some studies, total collection of ileal digesta is not possible. Thus, the equation for AID calculation is:

$$\text{AID, \%} = [1 - (M_{diet}/M_{ileal}) \times (AA_{ileal}/AA_{diet})] \times 100.$$

Where M_{diet} and M_{ileal} are the concentrations of index marker (g/kg, DM basis) in diet and ileal digesta, respectively; AA_{ileal} and AA_{diet} represent AA (g/kg, DM basis) concentrations in diet and ileal digesta, respectively.

The digesta collected from the ileum may contain both dietary undigested materials and endogenous protein and AA (Laplace et al., 1985). Thus, the ileal digestibility of AA calculated without considering endogenous AA losses is defined as AID. The AID of AA is not accurate in formulating diets, because the endogenous AA flow in the ileal digesta can lead to an underestimation of AA digestibility and lack of additivity in diets containing multiple protein sources (Fan et al., 1994), especially for ingredients with low protein content (Stein et al., 2005). Therefore, in order to accurately measure the ileal digestibility of AA for diet formulation, accurate determination of ileal endogenous AA losses (IAA_{end}) is necessary (Stein et al., 2007).

1.2.1 Endogenous losses of amino acids

The main sources of endogenous AA losses in animals are the proteins that are synthesized and secreted in the lumen of the gastrointestinal tract (GIT) in animals but not reabsorbed (e.g., digestive enzymes, mucin protein, and serum albumin), sloughed intestinal epithelial cells, and bacterial protein from the hind gut (Nyachoti et al., 1997a).

The IAA_{end} can be divided into two parts, basal (non-specific, or diet-independent) and specific endogenous losses (McDonald, 2011). As shown in Figure 1-1, the basal IAA_{end} is defined as the inevitable loss of AA in the GIT of animals, which is related to the amount of DM intake but otherwise unrelated to dietary composition (McDonald, 2011).

The specific endogenous loss is considered as the portion of endogenous AA flow, which is over and above basal IAA_{end} induced by the ingestion of diets of specific composition, such as protein level, fiber type, and anti-nutritional factors (Adedokun, 2007; Cowieson and Ravindran, 2007; Stein et al., 2007). The inclusion of a high concentration of protein in the diet can increase the specific endogenous loss of AA, because secretion of digestive enzymes in the GIT will be elevated in response to the high protein intake (Nyachoti et al., 1997b; Hodgkinson et al., 2000; Adedokun et al., 2008b). Likewise, fiber content and type can also affect the specific endogenous loss by changing the viscosity and passage rate of digesta in the small intestine, which can impact the secretion of mucin and epithelial cell turnover (Parsons et al., 1983; Mosenthin and Sauer, 1991). Anti-nutritional factors can also increase specific endogenous losses of AA. Cowieson and Ravindran (2007) reported that phytic acid can induce the secretion of specific endogenous losses of AA, and this increase of IAA_{end} can be ameliorated by the addition of phytase into the diet. This phenomenon can partly explain the effect of phytase in improving AID of AA in feed ingredients.

Ileal digestibility that is adjusted for IAA_{end} can be termed either as true ileal digestibility (TID), when the total IAA_{end} is corrected for in the calculation; or standardized ileal digestibility (SID), if corrected for basal IAA_{end} (Stein et al., 2007). For common feed ingredients such as corn, SBM, DDGS, and canola meal, SID of AA is additive in complete diets (Stein et al., 2005). The TID and SID calculation are:

$$\text{TID, \%} = [(\text{AA intake} - (\text{ileal AA output} - \text{IAA}_{end}))/\text{AA intake}] \times 100;$$

$$\text{SID, \%} = [(\text{AA intake} - (\text{ileal AA output} - \text{basal IAA}_{end}))/\text{AA intake}] \times 100.$$

If the AID is already estimated, the equations become:

$$\text{TID, \%} = \text{AID} + (\text{IAA}_{end}/\text{AA}_{diet}) \times 100;$$

$$\text{SID, \%} = \text{AID} + (\text{basal IAA}_{end}/\text{AA}_{diet}) \times 100.$$

Because of the relative ease of determining basal IAA_{end} and the additivity of SID in a complete diet, SID is accepted as the preferred measure of digestible AA requirement and content in swine diets. However, for poultry, the data of SID of AA in ingredients is not as copious as for swine. This is partly because the concept of SID was introduced after the latest version of NRC (1994) for poultry.

1.2.2 The determination of endogenous amino acids losses

The methodologies for measuring IAA_{end} were developed in the last few decades. These include use of nitrogen-free diet (NFD), fasting method (Green et al., 1987), highly digestible protein diet (Adedokun et al., 2007a), enzyme hydrolyzed protein diet (Yin et al., 2004), regression method (Eklund et al., 2015), homo-arginine diet (Nyachoti et al., 1997b), and ^{15}N isotope marked technique (Hess et al., 1998). Each of these methodologies have advantages and shortcomings. For example, some of these methods can be used for the determination of basal IAA_{end} (e.g., NFD and regression method), or total IAA_{end} (e.g., homo-arginine diet and ^{15}N isotope marked technique). However, in practical nutrition studies, it is technically difficult to completely distinguish the undigested dietary protein from the specific endogenous losses, and the procedure for separation of these fractions are both laborious and expensive. In addition, the SID of AA in feed ingredients is shown to be additive in a complete diet, which is important for diet formulation on digestible nutrient basis (Stein et al., 2005). Therefore, instead of TID, SID is an alternative for the expression of digestibility of AA in feed ingredients (Mosenthin et al., 2000; Jansman et al., 2002; Stein et al., 2007).

The equation for basal IAA_{end} estimation is:

$$\text{Basal } IAA_{end} = AA_{ileal} \times (M_{diet}/M_{ileal}).$$

The main concern with the NFD method is the physiological status of experimental animals (de Lange et al., 1989). Due to the deficiency of AA, the degradation of body proteins in animals fed with NFD is increased to release AA for maintaining normal biological functions, which will lead to a high level of dispensable AA, especially proline and glycine, in the ileal AA flow (Jansman et al., 2002). It has been reported that the predominant endogenous AA in IAA_{end} are glutamic acid, aspartic acid, leucine, and threonine (de Lange et al., 1989; Nyachoti et al., 1997b; Hess et al., 1998). However, when the NFD method is applied, it has been noted that the basal IAA_{end} are high in proline and glycine (Kim et al., 2009; Urriola et al., 2009a; Zhai and Adeola, 2011). This phenomenon sometimes results in SID estimates that are above 100% for those two AA.

Based on this consideration, it has been suggested that low inclusions of highly purified and digestible proteins (e.g., casein) should be added to NFD to ameliorate the AA deficiency and the abnormal physiological status of experimental animals (Adedokun et al., 2007a). The ileal AA flow should still be of endogenous origin, because it is assumed that the added protein is 100% digestible. Thus, the digestibility of casein should be measured within each study to ensure that the determined ileal AA flow is of endogenous origin. Cervantes-Pahm and Stein (2010) reported that SID of AA in casein for growing pigs is approximately 96% for most AA, measured with the direct method. However, Jezierny et al. (2011) and Eklund et al. (2015) indicated the SID of AA in casein is almost 100%, estimated by the regression method. The IAA_{end} in an animal fed casein might be increased with the inclusion of casein. However, if the basal IAA_{end} is determined by NFD, the estimated IAA_{end} could be lower than it is in a casein diet, which leads to a lower value of SID when the direct method is applied.

An alternative to supplementation of the NFD with a highly digestible protein source such as casein is the use of enzyme hydrolyzed protein (Golian et al., 2008). As the only source of protein in the diet is peptides of a (known) low molecular mass, endogenous protein can be separated from the undigested hydrolyzed protein by size separation. However, considering the potential stimulation of specific endogenous losses by the additional peptide, this method may overestimate the basal IAA_{end}. Another alternative method is the regression method (Fan and Sauer, 1997; Eklund et al., 2015) where a series of diets with increasing protein concentration can be used. The regression equation, which is a variation of Eq [6] is:

$$AID_C = (SID_C/100) \times AA_{diet} – \text{basal } IAA_{end}.$$

Where AID_C and SID_C are the content of digestible AA in experimental diet. AID_C and AA_{diet} are the dependent and independent variables, respectively. The slope provides an estimate of standardized ileal digestible AA in diet, and the intercept stands for basal IAA_{end}.

Jansman et al. (2002) reviewed previous data on IAA_{end} determined by the NFD, casein diet, and regression methods and summarized that the value of IAA_{end} generated from NFD was slightly lower than casein diet, except for proline and glycine, but similar to those of the regression method. Eklund et al. (2015) published data in cannulated pigs to determine the SID of AA in rapeseed meal using the regression method and casein to measure the basal IAA_{end}. Three levels of casein diet were used to estimate the SID of casein and the basal IAA_{end}. The results showed that the SID of AA in casein was close to 100% and the estimated basal IAA_{end}, which represents the intercept of the linear regression, was similar to the results generated from NFD method except for lower proline and glycine (Table 1-1). Similar results were reported in previous studies from the same group (Eklund et al., 2008; Jezierny et al., 2011; Eklund et al., 2014). Thus, the regression method with casein may be a useful method for determining basal IAA_{end} without the high level of proline and glycine in the ileal AA flow. However, the regression method is more laborious and requires more dietary treatments than the NFD method. Meanwhile, the intercept of the linear regression is obtained by extrapolation, which may not be statistically reliable due to the interval of regressor.

The average values of basal IAA_{end} of AA reported in pigs during the past decade are summarized in Table 2. As shown in the table, values from the casein diet are generally higher than values from the NFD or regression method. Excessive secretion of proline and glycine is observed in NFD and casein diets, but not in the regression method. Similar to previous observations of Jansman et al. (2002), the variation in determined basal IAA_{end} of dispensable AA is greater than that in indispensable AA.

Arginine, as a conditionally indispensable AA, varied more in the results of basal IAA_{end} between studies.

In poultry studies, the fasted cecectomized rooster model is also one of the methods for estimating IAA_{end} (Aldrich et al., 1997). However, it has been reported that the fasted cecectomized rooster model underestimates IAA_{end} (Parsons, 1986; Adedokun et al., 2008a; Adedokun et al., 2009) because excretion of basal IAA_{end} depends on DM intake. In addition, the unit IAA_{end} estimated from fasted cecectomized roosters is g/d, rather than based on DM intake. Thus, fasted animals cannot provide comparable estimates of basal IAA_{end} for determination of SID of AA in feed ingredients.

1.2.3 Factors in the determination of endogenous amino acids losses

As shown in Table 1-1, the amount and profile of basal IAA_{end} determined with NFD may vary between labs due to several factors. Even within the same lab, the determined value of basal IAA_{end} can vary among studies. Therefore, it is necessary to include NFD in every AA digestibility experiment, and to use the determined value of basal IAA_{end} for the calculation of SID. The recommended NFD formulation is shown in Table 1-3.

1.2.3.1 Experimental animal

Across poultry species (Table 1-2), the basal IAA_{end} (g/kg DM intake) in turkeys and ducks is higher than broiler chickens (Adedokun et al., 2007b; Adedokun et al., 2007c; Kong and Adeola, 2013b). Within the same species, the age of experimental animal can also impact the determination of basal IAA_{end} in digestibility studies. Adedokun et al.(2007b; 2007c, d) reported an age-related decrease in basal IAA_{end} in broiler chickens and turkeys. The basal IAA_{end} (g/kg DM intake) is higher in broiler chicks and turkeys on day 5 after hatch than on day 15, and is then maintained at similar level to d 21. It has also been found that the basal IAA_{end} was similar between 6-wk old broilers and 70-wk old layers and roosters (Ravindran and Hendriks, 2004). Thus, the use of birds that are at least 15 days old is preferred for determining AA digestibility in feed ingredients, especially when only AID of AA is investigated. The higher excretion of IAA_{end} may explain the lower digestibility of AA that is commonly observed in younger animals. The trend is similar in pigs (Hess and Sève, 1999; Hodgkinson et al., 2000; Leterme and Théwis, 2004). Weanling pigs secrete more endogenous AA compared with growing pigs. For growing pigs, the basal IAA_{end} (g/kg DM intake) tends to decrease with increasing BW (Pahm et al., 2008).

1.2.3.2 Feed intake

Although by definition, basal IAA_{end} should be constant on a g/kg DM intake basis, estimates from basal IAA_{end} determination can be influenced by amount of feed intake (Furuya and Kaji, 1992). Moter and Stein (2004) suggested that basal IAA_{end} (g/kg DM intake) decreased with an increase from energy intake at a maintenance level to ad libitum energy intake. The severe nutrient and energy deficiency may interfere with basic metabolism in the animal and result in a higher ileal basal IAA_{end}. Thus, feed intake should be maintained ad libitum or at least equivalent to 3x maintenance energy requirement for an accurate estimation of basal IAA_{end} (Moter and Stein, 2004). As long as the feed intake is maintained, number of meals per day does not appear to influence the basal IAA_{end} and SID determination in pigs (Chastanet et al., 2007).

1.2.3.3 Dietary fiber and mucin secretion

Mosenthin et al. (1994) indicated that the inclusion of pectin could decrease the AID of AA in SBM. This could be attributed to the effect of fiber on IAA_{end}. The type of dietary fiber may influence the pattern of IAA_{end} (Leterme and Théwis, 2004). The capacity of fiber to absorb water is critical for determining the viscosity of the digesta and the secretion of mucin in the small intestine. Therefore, the content and type of fiber could change the viscosity and rate of passage of digesta, as well as the dynamics and excretion of mucin (Nyachoti et al., 1997a; Piel et al., 2005). Usually, cellulose (solka-floc) will be added in the NFD to increase the bulkiness and maintain the physical texture of digesta, because the other ingredients in a NFD such as starch and dextrose are highly soluble components. Cellulose is added to NFD at between 4 and 5% of the diet among studies. The inclusion of cellulose should be maintained for consistency between studies. However, there is a dearth of studies investigating the effect of the level of cellulose on the determination of basal IAA_{end} using NFD in poultry and pigs.

1.2.3.4 Ingredient composition of the nitrogen-free diet

The basal IAA_{end} using the NFD method can be influenced by the ingredient composition of the NFD. It was observed in broiler chickens that the basal IAA_{end} was higher when corn starch was completely replaced by dextrose in the NFD, compared with partial or no replacement of corn starch (Kong and Adeola, 2013c). However, this effect is not observed in pigs (Kong et al., 2014b). Cervantes-Pahm and Stein (2008) observed different SID of AA in SBM in semi-purified diets with or without added soy oil. This result may suggest that oil content might have an effect on basal IAA_{end}. However, there is a lack of information on the effect of NFD oil content on basal IAA_{end}. Favero et al. (2014) indicated that the type of index marker can also affect the result of basal IAA_{end}.

In this particular study, acid-insoluble ash (AIA), chromic oxide, and titanium dioxide were added in NFD. The results showed that basal IAA_{end} calculated based on chromic oxide index was lower than those from AIA or titanium dioxide.

1.2.4 Implications

SID of AA is widely accepted as a method for expressing AA digestibility in diet formulation and AA requirement. The accurate measurement of basal IAA_{end} is critical to the determination of SID. Because the estimation of basal IAA_{end} can be affected by experimental animals, diets, and methods, we suggest the need to determine the basal IAA_{end} in each AA digestibility study rather than use of an average value to calculate SID. Using NFD is the preferred method because of its simplicity and the definition of basal IAA_{end}. Although the basal IAA_{end} estimate for a dispensable AA is influenced by the physiological state of the experimental animal, the SID of most AA in feed ingredients based on the NFD method is additive in a complete diet. Due to the influence of ingredient composition on the determination of basal IAA_{end}, standard NFD formulation should be applied for consistency among studies. Besides the NFD method, the regression method using casein is an alternative to estimate basal IAA_{end} for calculating SID of AA.

Additional studies are needed to understand the factors that affect the determination of basal IAA_{end}. Dietary composition of NFD, such as fiber and oil content, need to be investigated for standardizing the composition of NFD used for the determination of basal IAA_{end}.

1.3 Phosphorus

In earlier studies from the last century, the digestibility and retention of P in feed ingredients has also been referred to availability (Bayley et al., 1968). Digestible P was expressed as available P in some references (Nelson et al., 1968; Simons et al., 1990). The terminology of "P availability" and "available P" do not specifically refer to apparent or true digestibility of P. This may lead to confusion for the diet formulation in the swine and poultry industry. The availability of P can also be confused with the concept of bioavailability of P in feed ingredients, which is determined by using the slope-ratio assay with a reference ingredient (Burns and Baker, 1976; Tagari et al., 1981). For those reasons, it is more precise to use the terminology of apparent, true, or standardized digestibility of P to investigate the digestion and retention of P in swine and poultry.

1.3.1 The methodology for estimating phosphorus digestibilites in feed ingredients

The digestibility of P in feed ingredients can be measured on either total tract or ileal digestible basis. For growing pigs, to determine the apparent total tract digestibility (ATTD) or retention of P, the measurements of dietary P intake and fecal P output are needed. For poultry species, the output of excreta is a mixture of intestinal digesta and renal excretion. Therefore the terminology is apparent total tract retention (ATTR) if the excreta is investigated for P output.

The total collection approach can be applied to determine the ATTD of P in feed ingredients for swine and poultry (Adeola, 2001). In this approach, feed intake should be recorded, and the feces or excreta output need to be collected completely during a period of time. The equation that is used to calculate the ATTD is:

$$\text{ATTD} = 100 \times (\text{Nutrient}_{\text{I}} - \text{Nutrient}_{\text{O}}) / \text{Nutrient}_{\text{I}},$$

where $Nutrient_I$ is the amount of nutrient intake (g/d) and the $Nutrient_O$ is the quantity of nutrient output (g/d) in feces. In studies using growing pigs, the marker to marker technique can be applied to indicate the starting and ending point of the total collection. As described by Zhai and Adeola (2012), after an adaptation period of experimental diet, which is typically 5-7 d, an indigestible marker with recognizable color should be added to the first meal during the total collection period. The total collection of feces should be started from the time point that the color appeared in the feces. The fecal sample with the color should be collected. With the progression of the experiment, the color of the marker should fade away in the fecal samples. After the total collection period, another meal with indigestible marker should be fed to the animal. The total collection of fecal samples should be continued until the feces with the color of the second marker has been excreted. The fecal sample with the color should not be collected. Following this marker to marker approach, the fecal sample collected during the total collection period is exactly corresponding to the amount of feed intake during this period. For poultry species with relatively shorter GIT and excretion time after feed ingestion, the birds can be put through a fasting period to empty the GIT. Then the ending of the total collection can be defined as a prolonged collection after the feed withdrawal (Ravindran et al., 2000). Another technique that can be applied in poultry is the precision-fed and total collection method (Johns et al., 1986). In this kind of experiment, roosters are usually used rather than broiler chickens, because the body and GIT of roosters are more mature to undergo the experimental operations such as force feeding.

Ileal samples can also be used to estimate P output flow in the small intestine. The techniques that are used to collect ileal samples are similar to those for the investigation of AA digestibility, as we have already described in earlier sections. The index method can be applied to investigate the AID of P in feed ingredients for swine and poultry, due to the difficulties with the total collection of ileal digesta. The equation for the calculation of AID of P is similar to the determination of AID of AA.

Generally speaking, the digestibility of P can be determined by either the direct method or through the difference method. In the direct method, a semi-purified diet is formulated with the candidate ingredient, where all the P in the experimental diet is solely contributed by the candidate ingredient. The ATTD or AID of P determined using this diet can represent the digestibility of P in the candidate ingredient. In the application of the difference method, or the indirect method, a basal diet is needed in the experiment (Pereira and Adeola, 2016). Then the test diet is formulated using the candidate ingredient to proportionally replace the basal diet. The equation to calculate the digestibility using the difference method is (Adeola, 2001):

$$A, \% = 100 \times [(T \times T_P) – (B \times B_P)] / A_P,$$

where T, B, and A is the digestibility (%) of the component in the test diet, basal diet, and candidate ingredient, respectively; T_P, B_P, and A_P is the proportion (%) of the component in the total diet that is contributed by the test diet, basal diet, and candidate ingredient, respectively; $T_P = B_P + A_P = 100\%$.

For most of the ingredients that can be a significant P source in swine and poultry diets, such as oil seed meals and distillers by-products, it is easy to formulate a semi-purified diet to determine the digestibility of P by using the direct method. Therefore, most of the studies focused on P digestibility in feed ingredients were conducted by using the direct method, though it can be determined by both direct and indirect methods.

1.3.2 Ileal and total tract digestibility of Phosphorus

The small intestine is the major site for P digestion and absorption. By the end of the ileum, approximately 85-95% of the absorption of P has been completed (Rutherfurd et al., 2014). Generally speaking, the measurements of ATTD and AID of P in common feed ingredients are not statistically different from each other. In previous studies (Shen et al., 2002; Johnston et al., 2013; Rutherfurd et al., 2014), although there was a numerical increase in the digestibility of P from AID to ATTD, the difference was not statistically significant. These results indicate that the large intestine does not play an important role in net P absorption. The digestibility and requirement of P for swine and poultry can be expressed on either the total tract or the ileal digestible basis. However, microbial activity in the hindgut can have impact on the degradation of phytate in the digesta (Jongbloed et al., 1992; Angel et al., 2005). Thus, ileal digestibility is more accurate if the objective of the study is related to phytate degradation. In broiler chickens, it is reported that P absorption has finished by the end of the proximal one-third of the ileum (Rodehutscord et al., 2012). Therefore, ileal samples from the distal two-thirds should be collected for the determination of P digestibility.

1.3.3 Terminologies of P digestibilites

Similar to the components of AA flow in the GIT, P flow also consists of undigested dietary P and endogenous P loss (EPL). The composition of the P flow in the GIT is shown in Figure 1-2. The sources of EPL include the turnover of mucosal cells, salivary juice, gastric juice, biliary juice, and pancreatic secretions (Fan et al., 2001). It was reported that the EPL in growing pigs increased along with the BW (Pettey et al., 2006). As discussed in the previous section (Figure 1-1), the AA_{end} can be divided into basal AA_{end} and specific AA_{end}. Likewise, the total EPL can be defined as the combination of basal and specific EPL (Petersen and Stein, 2006). Thus, the total tract digestibility of P can also be defined as apparent, standardized (STTD), and true (TTTD) total tract digestibility (retention, STTR and TTTR in broiler chickens), depending on how the EPL are accounted for. The AID and ATTD of P do not take the EPL into account. The estimation of P digestibility is somehow underestimated. Therefore, the true or standardized digestibility of P are more accurate in the expression of digestible P content in feed ingredients, dietary formulation, and expression of dietary requirements in swine and poultry. Previous research has indicated that the TTTD of P in corn and SBM are additive for growing pigs (Zhai and Adeola, 2013).

1.3.4 The regression method and P digestibilites

The regression method can be applied to determine the TID or TTTD of P in feed ingredients (Fan et al., 2001; Shen et al., 2002). Determination of total EPL in swine and poultry is relatively difficult compared with the estimation of AA_{end}. The methods that can be used to separate the EPL and undigested dietary P in the P output are very limited. For this reason, the regression method is preferred in the investigation of TID or TTTD of P in feed ingredients.

In the determination of P digestibility by using the regression method, a set of diets with increasing concentration of the candidate ingredient is needed. Generally, the dietary P intake gradients should be formulated at marginal deficiency level. It is critical to formulate the experimental diet in such a way that the increasing P intake should be solely contributed by the candidate ingredient. Thus, theoretically:

$$\text{True digestibility (or retention) of P} = \Delta P_D / \Delta P_I,$$

where ΔP_D means the increased digested (or retained) P; ΔP_I stands for the increased P intake. In the linear regression analysis, the estimation of the slope is the coefficient of true digestibility (or retention) of P, when the P digested is regressed against P intake. The regression model usually is:

$$P_D = (C_{TD} \times P_I) + \text{Intercept},$$

where P_D is the digested P (g/DMI); P_I is the dietary P intake (g/DMI); and C_{TD} is the coefficient of true digestibility (or retention). When the candidate ingredient is the only P source in the experimental diets, the intercept can be considered as the estimation of EPL. In some situations, the regression model can also be expressed as:

$$P_O = (C_{TI} \times P_I) + \text{Intercept},$$

where P_O is the P output and C_{TI} stands for the coefficient of true indigestibility of P. Then the coefficient of true digestibility (or retention) can be calculated by:

$$C_{TD} = 1 - C_{TI}.$$

Previous studies suggested that type of basal diet does not affect regression-derived estimates of P digestibility (Liu et al., 2014b; Shastak et al., 2014). In the application of the regression method to determine the true P digestibility and retention, the dietary P concentrations in the experiment is critical for accurate estimation. It is reported that the amount of digested P is linearly increased by the increasing dietary P intake in a wide range below the requirement (Stein et al., 2008). However, for a wider range of P intake, the response of P retention can be described by a monomolecular model (Schulin-Zeuthen et al., 2007). The statistic model for a monomolecular function is:

$$Y = a + b\,(1-e^{-cx}),$$

where Y is the dependent variable that stands for the retention of P, X is the independent variable that means the P intake; and c is the slope. The monomolecular model of P retention is shown in Figure 1-3. As presented, the response of P retention to dietary P concentration in P deficient conditions can be considered as a linear pattern. Thus, linear regression analysis can be applied to estimate the retention efficiency of P within this range. At a marginal deficiency, the increment of P retention is in a non-linear pattern and therefore leads to a decreasing slope. Once the plateau of P retention is reached, the slope becomes zero. For this reason, it is necessary to formulate the experimental diets in a P deficient situation, in order to apply the linear regression analysis within the suitable range. To confirm the linear pattern, the increasing P digestion (or output) in response to the dietary P concentrations should be tested in the statistical analysis. If the quadratic response of P digestion (or output) is observed, it should be viewed cautiously, because the estimation of the slope for the linear regression might be affected (Mutucumarana et al., 2015a).

Although it is reported that the response of P digestion is in a linear pattern to a wide range of dietary P concentrations (Stein et al., 2008), it is very important to control the inclusion rate of the candidate ingredient in the experimental diets, especially for ingredients containing anti-nutritional factors, such as phytate or high fiber content. The content of anti-nutritional factors may negatively affect the digestion of P in the GIT (Leytem and Thacker, 2010; Swiech et al., 2012). As a result, the estimation of the slope for the linear regression analysis might be affected, because of decreased P digestion in the high inclusion rate diets.

Notably, during the comparison of two slopes derived from the regression method in the same study, such as to compare the digestibility of P in two different ingredients or to test the effect of an enzyme product on the TID or TTTD of P, the analysis of covariance (ANCOVA) should be applied. The statistical model for ANCOVA is:

$$P_D = \text{Treatment} \;\; P_I \;\; \text{Treatment}*P_I,$$

where P_D is the digested P (g/kg DMI); Treatment is coded as a dummy variable (0 for one candidate ingredient or without enzyme supplementation; 1 for the other candidate ingredient or with enzyme supplementation); P_I is dietary P intake (g/kg DMI); and Treatment*P_I is the interaction. The difference between the two slopes can be tested within this statistical model.

1.3.5 <u>The estimation of endogenous P losses</u>

As described in Figure 1-2, the P flow in the GIT can be divided to undigested dietary P and EPL. Thus, similar to the terminologies in the determination of AA digestibility, the digestibility of P can be named as AID, ATTD, SID, STTD, TID, or TTTD, depending on how the EPL was accounted for during the determination. Although the amount of EPL is relatively small, the determination of apparent P digestibility and retention can be affected by the EPL (Fan et al., 2001; Shen et al., 2002). It is suggested that the EPL should be accounted for to eliminate the impact of dietary P concentration on the determination of P digestibility (Fan et al., 2001).

The total EPL can also be considered as a combination of basal EPL, which is dependent on the DM intake, and the specific EPL. The radioactive isotope of P can be used to determine the TID or TTTD of P and the total EPL (Whittemore and Thompson, 1969). However, the application of isotopic methods is difficult and expensive. For these reasons, the isotopic method is not widely applied to determine the TID and TTTD of P. It is relatively easy to estimate the basal EPL by using a P-free diet (Petersen and Stein, 2006). A P-free diet that is typically formulated based on cornstarch, sucrose, and gelatin can be included with the experimental diets to determine the basal EPL for the calculation of SID or STTD of P. The equations for the estimation of the basal EPL, SID, and STTD of P are the same as the equations previously described in the calculation of AA digestibilites.

The determined EPL in growing pigs and broiler chickens reported in previous studies is summarized in Table 1-4 and 1-5. It is indicated in previous literature that the basal EPL in swine is approximately 190 mg/kg DMI (NRC, 2012). Generally speaking, the ileal EPL is numerically greater compared with fecal EPL. This phenomenon suggests that the large intestine may play a role in the reabsorption of endogenous P. The estimated value of EPL is numerically greater in those studies conducted on ileal cannulated pigs. This increased EPL might be a result of the cannulation surgery and the continuous sampling from the cannula during the experiments.

The role of P in animal nutrition is critical. Therefore, there is also a concern of the abnormal physiological status which might affect the estimation of EPL when the P-free diet method is applied. The EPL in swine and poultry can also be measured by the regression analysis (Fan et al., 2001). In the linear regression model, the estimation of EPL can be derived by extrapolating the P intake to zero (Dilger and Adeola, 2006a, b; Mutucumarana et al., 2014a). By comparing the results of EPL determined by the regression method and P-free diet, the variation from the regression method is greater among studies (Table 1-4 and 1-5). There are several key points that should be noted when the regression method is applied to determine the EPL in growing pigs and broiler chickens. When the regression model is used to predict the dependent variable, it is not reliable to estimate by extrapolating the independent variable out of the interval, due to the limitation of the statistical methodology of regression analysis. This extrapolation can lead to high variation in the standard error of the estimated intercept in the regression analysis. It is possible that the determined EPL was not statistically different from zero when the regression method was applied (Liu et al., 2014a). As mentioned in the previous section (Figure 1-3.), the retention of P, in fact, is more accurate to be described by the monomolecular model rather than the linear model (Létourneau-Montminy et al., 2012). The derivative should be greater when the P intake is close to zero in the monomolecular model. Thus, the intercept of the linear regression may underestimate the EPL. For this reason, the lowest dietary P level in the graded P levels in the experimental diets should be close to zero, when the regression method is used to estimate EPL in growing pigs and broiler chickens.

To ameliorate the abnormal physiological status caused by the semi-purified experimental diet, high digestible nutrient supplementation, such as casein, was suggested to be added to the experimental diets (Iyayi et al., 2013). It was indicated in previous studies that the supplementation of casein in the basal diet does not affect the digestibility of P (Liu et al., 2014a). In this scenario, the regression method can still be used to determine the TID or TTTD of P, if the increased P intake and P digestion are solely contributed by the candidate ingredient. But, the regression method cannot provide accurate estimation of EPL if the basal diet contained P. In the regression method, the EPL is estimated by the extrapolation of the linear regression equation to zero P intake. Thus, it is assumed that the slope is a constant from zero P intake to the lowest dietary P level in the experimental diets. It is indicated that the intercept of the linear regression will be affected by the P source in the basal diet (Liu et al., 2014a).

The effect of P source in the basal diet on the estimation of EPL by the using regression method is presented in Figure 1-4. As described in the previous section, the regression model used to determine the TID or TTTD of P in the candidate ingredient is:

$$P_D = (C_{TD} \times P_I) + \text{Intercept}.$$

where P_D is the digested P; P_I is the dietary P intake; and C_{TD} is the coefficient of true digestibility. If the candidate ingredient is the only P source in the diet, the equation can be expressed as:

$$P_D = (C_{TD} \times P_I) - \text{EPL}.$$

The estimated EPL is equal to the negative value of the intercept. If there is P source in the basal diet, the equation can be decomposed to:

$$P_D = [C_{TDC} \times P_{IC} + C_{TDB} \times P_{IB})] - \text{EPL},$$

where P_{IC} and P_{IB} is the P intake contributed by the candidate ingredient and the basal diet, respectively; C_{TDC} and C_{TDB} is the coefficient of true digestibility of P in the candidate ingredient and the basal diet, respectively. Then, the equation can be transformed as below:

$$P_D = C_{TDC} \times (P_I - P_{IB}) + C_{TDB} \times P_{IB} - EPL$$

$$P_D = C_{TDC} \times P_I - C_{TDC} \times P_{IB} + C_{TDB} \times P_{IB} - EPL$$

$$P_D = C_{TDC} \times P_I - (C_{TDC} - C_{TDB}) \times P_{IB} - EPL.$$

In this equation,

$$\text{Intercept} = -EPL - (C_{TDC} - C_{TDB}) \times P_{IB},$$

which is a still a constant. Therefore, this model can still be applied to determine the TID or TTTD of P in the candidate ingredient. However, the estimated EPL from this model is equal to [EPL + (C_{TDC}–C_{TDB}) × P_{IB}]. Only in the situation that C_{TDC} is equal to C_{TDB}, which means the true digestibility of P in the basal diet is equal to the candidate ingredient, the intercept of this model can be used to estimate the EPL; when the true digestibility of P in the basal diet is greater than the candidate ingredient, the EPL will be underestimated; on the contrary, the EPL will be overestimated if the true digestibility of P is greater in the candidate ingredient.

1.3.6 Factors impacting phosphorus digestibility

The digestion and absorption of P in the GIT is associated with several factors from both the animal and the diet composition. In the GIT of swine and poultry, the main site of P absorption is the jejunum.

1.3.6.1 The transport of P in the small intestine

The transport of P across the epithelial cells is dependent upon passive diffusion and active transport via sodium-phosphate co-transporter IIb (NaPi-IIb) (Huber et al., 2006; Saddoris et al., 2010). The regulation of the NaPi-IIb gene expression in the small intestine is dependent on the concentration of free phosphate in the lumen of the small intestine (Huber et al., 2015), which is in a pattern of adaptive regulation. The gene expression of NaPi-IIb can be upregulated by the low P concentration in the small intestine (Olukosi et al., 2011; Nie et al., 2013). Thus, the factors that may affect the concentration of free phosphate, such as the presence of phytate, in the GIT may also influence the absorption of P.

1.3.6.2 <u>The presence of phytic acid and the additional phytase in feed</u>

Phytic acid (*myo*-inositol hexaphosphate; IP_6) is the main storage form of P in plant tissues, especially in bran and seeds (Kasim and Edwards, 1998). As a result, the majority of P in plant origin feed ingredients is in the form of phytic acid. In the molecule of phytic acid, there are six phosphate groups attached on one inositol ring. Thus, the concentration of P in phytic acid is 28.2% (Selle et al., 2009). Phytate is the salt of phytic acid. The digestion of phytate is limited in non-ruminant animals due to the lack of enzyme to release the phosphate group (Douglas et al., 2000; Hanne Damgaard, 2000). For this reason, the addition of inorganic P in animal diets is necessary to meet the P requirement. Although the concentration of phytic acid is almost zero in the feces of growing pigs, the majority of the degradation takes place in the hindgut by microbial activity (Angel et al., 2005). The degradation of phytate in the hindgut has little contribution to whole body P nutrition, because the main site of P abortion is the small intestine (Rutherfurd et al., 2014).

The presence of phytate may not only reduce the digestibility of P but also can impair the digestibility of other minerals and even AA, because the negative charge of phosphate on phytate may chelate cations (Ravindran et al., 1999; Selle et al., 2012; González-Vega et al., 2015). For this reason, the presence of phytate can also increase the endogenous losses of nitrogen and amino acids, as well as trace minerals (Cowieson et al., 2004). The supplementation of phytase in the diet can ameliorate the negative effect of phytate on the digestion of P and other nutrients in swine and poultry (Jongbloed et al., 1992; Adeola et al., 1995; Olukosi et al., 2013), which is of great importance in reducing the P output to the environment and feed cost.

Phytase (*myo*-inositol hexakisphosphate phosphohydrolases) is a family of hydrolases that are capable of catalyzing the stepwise hydrolysis of phytic acid (Adeola and Cowieson, 2011). Depending on which one of the six phosphate groups the enzyme initiates hydrolysis, common feed related phytase can be classified as 3-phytase and 6-phytase. Among these two kinds of phytase, 3-phytase typically originates from plants, while 6-phytase is typically from microorganisms (Adeola and Cowieson, 2011). The effect of phytase on improving the digestibility of P in growing pigs and broiler chickens is widely confirmed (Adeola et al., 2004; Almeida and Stein, 2012; Adedokun et al., 2015). This improvement of P digestibility was observed in cereal grains such as corn and wheat, as well as oil seed meals such as SBM, canola meal, copra meal, and peanut flour (Iyayi et al., 2013; Almaguer et al., 2014; Maison et al., 2015). In distiller's dried grains with solubles (DDGS), the digestibility of P is relatively higher compared with the corresponding grain, because the microbial fermentation procedure during the production of ethanol already degrades a part of phytate in the grain. The addition of phytase can still improve the digestibility of P in DDGS in growing pigs and broiler chickens (Martinez-Amezcua et al., 2006). By hydrolyzing the phytate in feed, the phytase may also improve the digestibilites of other nutrients that can be chelated by phytic acid. The addition of phytase in the diet can improve the digestibilites of minerals such as Ca and Mg, in growing pigs and broiler chickens (Adedokun et al., 2015; González-Vega et al., 2015). Compared with the effect of phytase on the digestibility of P and Ca, the impact on AA digestibilites is not consistent among studies. It is reported that phytase supplementation increased AA digestibilites in broiler chickens (Ravindran et al., 2001; Adedokun et al., 2015). However, studies also suggesting that phytase exerts little effect on the digestibilites of AA in swine and poultry (Omogbenigun et al., 2003; Nitrayova et al., 2006; Cervantes et al., 2011; Morales et al., 2012).

The amount of released P from phytate by phytase is in a dose dependent pattern (Adeola and Cowieson, 2011). For this reason, in swine and poultry diets based on corn and SBM, it is suggested that the equivalency of phytase to the inorganic P source can be summarized to predict the P released by phytase (Kornegay and Qian, 1996; Jendza et al., 2006). However, the condition (such as animals and dietary composition) of the equivalency estimation should be specified. It is reported that the response of P digestibility to dietary phytase supplementation is greater in growing pigs and sows compared with piglets (Kemme et al., 1997). This may be due to the larger volume of the GIT in growing pigs and sows provides the environment for the enzyme.

The hydrolysis of phytic acid is a stepwise reaction that needs six steps to release all the phosphate groups from the inositol molecule. It is difficult to release all the P from phytate (Rutherfurd et al., 2014). However, there is evidence that large dosages of phytase may further increase the hydrolysis of phytate to inositol (Walk et al., 2014). Typically, the dosage of phytase in a practical diet for growing pigs or broiler chickens can be at 500 or 1000 FTU/kg. In the past decade, with the development of phytase producing technology which decreased the price of commercial phytase products and the potential benefits of phytase super-dosing, there are several studies working on this topic (Cowieson et al., 2011; Zeng et al., 2014; Manobhavan et al., 2015). Although the additional phytase supplementation may not further increase the digestibility of P (Walk et al., 2013), it is indicated that phytase dosage above the recommended concentration (added phytase at 500, 1000, 1500, and 2000 FTU/kg) linearly increased the growth performance of growing pigs (Bradley, 2014). The effect of phytase super-dosing may be attributed to the breakdown of phytate complexes (Walk et al., 2013).

1.3.6.3 Dietary calcium concentration

The absorption and utilization of P in animals is closely related to the Ca nutrition (Wasserman, 1981). The mineralization of bone requires both P and Ca (Garlich et al., 1975). As the main storage pool of Ca and P, the ratio between Ca and P is 2:1 in bone tissues. During the digestion and absorption of P, the concentration of Ca plays critical role, because the Ca cation can chelate with phytate and lead to a stable complex that reduces the potential for phosphate to be hydrolyzed from phytate (Selle et al., 2009; Cowieson, 2010; Symeou et al., 2012). It was demonstrated that the solubility of Ca in the duodenum and jejunum of broiler chickens was only 11%, indicating Ca-phytate aggregation (Pang and Applegate, 2007). As a result, the dietary concentration of Ca may affect the degradation of phytate as well as the efficacy of dietary supplementation of phytase (Tamim and Angel, 2003; Tamim et al., 2004). In addition, Ca can also bind with endogenous phosphate in the GIT which can lead to decreased reabsorption of endogenous phosphate (Rutherfurd et al., 2002; Rutherfurd et al., 2004). Therefore, an increased Ca to P ratio may limit the digestion and utilization of P. The TTTD of P in SBM for broiler chickens was determined to be greater at Ca to P ratio of 0.8, compared with 1.2, 1.6, and 2.0 (Liu et al., 2013). On the other hand, a low Ca to P ratio may impair bone mineralization (Létourneau-Montminy et al., 2010). Therefore, the Ca to P ratio should be controlled in swine and poultry diets due to its important role on P and Ca nutrition status. In practical diets for growing pigs, the Ca to total P ratio should be maintained at about 1.2:1, and the Ca to STTD P ratio should be approximately 2:1 (NRC, 2012).

1.3.7 Implications

To eliminate the effect of dietary P concentration on the determination of AID and ATTD (or ATTR) of P in feed ingredients, the SID, STTD, TID, TTTD or TTTR of P should be determined for growing pigs and broiler chickens. The regression method can be applied to determine the TID, TTTD, and TTTR of P in feed ingredients. It is important to formulate the diets with P concentration lower than the marginal deficient concentration to obtain an accurate estimation of true P digestion and retention due to the monomolecular response of the P digestion and retention to dietary P intake. The response of digested or retained P to the increasing dietary P concentration should be investigated during the application of the regression method. It should be noted that the estimation of TID, TTTD, or TTTR might be affected if the response is in a quadratic pattern. The regression method is not recommended to estimate the EPL in growing pigs and broiler chickens, because the estimates derived from extrapolation of the linear regression is statistically unreliable. In addition, the regression method cannot be applied to estimate EPL if the basal diet contains a P source. The P-free diet is preferred to estimate the basal EPL and the calculation of SID or STTD of P in feed ingredients.

1.4 The relationship between phosphorus and amino acids digestion

By summarizing previous reports on the whole body composition in growing pigs, it is indicated by NRC (2012) that the whole body composition of P and N are correlated in growing pigs. This correlation was used to estimate the P requirement in NRC (2012), associated with the protein deposition model of growing pigs. The relationship of whole body composition of N and P may suggest that the deposition of N and P are simultaneous. This phenomenon indicates that the digestion and retention of N and P may be quantitatively related. However, there is a lack of research that focus on this assumption. Further studies on this topic is necessary.

1.5 Summary

Literature review briefly introduced the methodology of the determination of the digestibility of AA and P in feed ingredients for growing pigs and broiler chickens. The definition and terminology of AA and P digestibility in poultry and swine nutrition is discussed. Compared with AID and TID, SID of AA is recommended for the expression of digestible AA contents of feed ingredients and for describing nutritional requirements of poultry and swine. To determine the SID of AA, total ileal flow of AA should be corrected for basal IAA_{end}. Although this AA deficiency may affect the estimate of basal IAA_{end} for dispensable AA, especially proline and glycine because of the degradation of body protein, the NFD method is still the most widely used method for basal IAA_{end} measurements. To improve the accuracy of estimating the SID of AA in feed ingredients, it is suggested that a NFD treatment should also be included in individual studies used to determine basal IAA_{end}. Similar to the determination of AA digestibility, a P-free diet can also be used to determine the basal EPL and calculate the SID and STTD of P. The regression analysis can be used to determine the true digestibility and retention of P in growing pigs and broiler chickens, but is not preferred to estimate the EPL. The digestion and retention of protein and P might be correlated with each other in growing pigs and broiler chickens.

1.6 Objective

The objective of this study was to investigate the methodology of the determination of the digestibility of AA and P in growing pigs and broiler chickens. The additivity of the AID and SID of AA for different feed ingredients was determined. The regression method was used to determine the TTTD of P for swine. The effect of dietary N and P on the digestion and retention of these two nutrients and the quantitative relationship between N and P retention was investigated.

1.7 References

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Table 1-1 Estimated basal endogenous loss of AA and the ratio of each AA to Lys in pigs from different studies

	Stein et al. (2005)		Zhai and Adeola (2011)		Xue et al. (2014)		Eklund et al. (2015)	
Method	Nitrogen-free diet		Nitrogen-free diet		Nitrogen-free diet		Regression method with casein diets	
Initial BW	92.1 ± 3.19 kg		47.1 ± 1.0 kg		61.3 ± 5.5 kg		22 ± 1 kg	
Item	Endogenous loss, g/kg DMI	Ratio to Lys[1]	Endogenous loss, g/kg DMI	Ratio to Lys	Endogenous loss, g/kg DMI	Ratio to Lys	Endogenous loss, g/kg DMI	Ratio to Lys
Nitrogen	2.26	-	-	-	2.32	-	1.92	-
Indispensable AA								
Arginine	0.45	92	0.44	104	0.54	111	0.4	80
Histidine	0.19	39	0.17	39	0.19	38	0.2	40
Isoleucine	0.42	86	0.27	63	0.33	68	0.4	80
Leucine	0.66	135	0.46	108	0.56	114	0.6	120
Lysine	0.49	100	0.43	100	0.49	100	0.5	100
Methionine	0.12	24	0.07	15	0.09	19	0.1	20
Phenylalanine	0.39	80	0.27	63	0.32	66	0.4	80
Threonine	0.55	112	0.42	100	0.49	101	0.7	140
Tryptophan	0.10	20	0.10	24	0.11	23	0.2	40
Valine	0.53	108	0.39	91	0.47	97	0.5	100
Dispensable AA								
Alanine	0.67	67	0.45	108	0.51	105	0.5	100
Aspartic Acid	0.93	93	0.64	152	0.83	169	0.9	180
Cysteine	0.28	28	0.14	32	0.19	38	0.2	40
Glutamic Acid	1.21	121	0.74	173	1.02	209	1.0	200

Table 1-1 Estimated basal endogenous loss of AA and the ratio of each AA to Lys in pigs from different studies (Continued)

	Stein et al. (2005)		Zhai and Adeola (2011)		Xue et al. (2014)		Eklund et al. (2015)	
Method	Nitrogen-free diet		Nitrogen-free diet		Nitrogen-free diet		Regression method with casein diets	
Initial BW	92.1 ± 3.19 kg		47.1 ± 1.0 kg		61.3 ± 5.5 kg		22 ± 1 kg	
Item	Endogenous loss, g/kg DMI	Ratio to Lys[1]	Endogenous loss, g/kg DMI	Ratio to Lys	Endogenous loss, g/kg DMI	Ratio to Lys	Endogenous loss, g/kg DMI	Ratio to Lys
Glycine	1.06	106	1.05	260	1.16	237	0.6	120
Proline	2.47	247	3.10	745	3.17	651	0.7	140
Serine	0.49	49	0.35	82	0.45	91	0.6	120
Tyrosine	0.35	35	0.21	51	0.25	51	-	-
Total AA	11.36	-	9.67	-	11.39	-	8.50	-

[1] Ratios are calculated by dividing endogenous loss of each AA by the endogenous loss of lysine and multiplying by 100.

Table 1-2 Mean of determined basal endogenous losses of AA (g/kg DM intake basis) with different methods in some previous studies in past decade (2005-2015)

Species	Pigs			Broilers			Turkeys			Ducks
Method	Nitrogen-free diet[1]	Casein diet[2]	Regression[3]	Nitrogen-free diet[4]	Casein diet[5]	Regression[6]	Nitrogen-free diet[7]	Casein diet[8]	Regression[9]	Nitrogen-free diet[10]
N	33	4	3	10	5	6	2	2	2	2
CP	17.28	19.14	12.47	11.29	-	-	-	-	-	36.30
Indispensable AA										
Arginine	0.59	0.73	0.43	0.39	0.22	0.18	0.27	0.31	0.27	1.49
Histidine	0.17	0.37	0.23	0.18	0.13	0.07	0.15	0.17	0.14	0.51
Isoleucine	0.30	0.46	0.43	0.37	0.38	0.20	0.25	0.39	0.26	1.26
Leucine	0.50	0.61	0.63	0.56	0.37	0.27	0.41	0.49	0.40	2.15
Lysine	0.40	0.47	0.50	0.39	0.30	0.15	0.27	0.35	0.24	1.18
Methionine	0.11	0.35	0.13	0.11	0.10	0.06	0.09	0.12	0.10	2.23
Phenylalanine	0.32	0.32	0.43	0.37	0.33	0.28	0.26	0.28	0.24	1.21
Threonine	0.52	0.73	0.70	0.60	0.45	0.34	0.44	0.47	0.38	1.43
Tryptophan	0.13	-	0.17	0.09	0.09	0.08	0.07	0.07	-	0.24
Valine	0.46	0.59	0.57	0.51	0.39	0.24	0.40	0.49	0.37	1.76
Dispensable AA										
Alanine	0.57	0.68	0.57	0.39	0.28	0.19	0.31	0.37	0.29	1.33
Aspartic Acid	0.75	1.01	0.93	0.73	0.56	0.36	0.56	0.68	0.53	2.34
Cysteine	0.17	0.67	0.25	0.41	0.16	0.14	0.21	0.23	0.21	1.04
Glutamic Acid	0.94	1.70	1.33	0.98	1.11	0.41	0.70	1.14	0.52	3.03
Glycine	1.46	1.45	0.70	0.47	0.28	0.22	0.33	0.36	0.51	1.64
Proline	4.95	6.20	0.67	0.50	0.38	0.24	0.40	0.46	0.35	1.87
Serine	0.65	0.90	0.77	0.56	0.61	0.33	0.39	0.57	0.38	1.64
Tyrosine	0.35	0.25	-	0.30	0.17	0.11	0.19	0.22	0.18	0.70
Total AA	13.30	17.42	9.37	8.40	6.61	4.04	6.64	7.75	5.95	31.05

[1]Means of previous studies (Bohlke et al., 2005; Stein et al., 2005; Gottlob et al., 2006; Stein et al., 2006; Mateo and Stein, 2007; Pedersen et al., 2007; Widmer et al., 2007; Pahm et al., 2008; Yin et al., 2008; Baker and Stein, 2009; Kim et al., 2009; Urriola et al., 2009b; Cervantes-Pahm and Stein, 2010; Cozannet et al., 2010; Jacela et al., 2010; Almeida et al., 2011; González-Vega et al., 2011; Jacela et al., 2011; Ren et al., 2011; Zhai and Adeola, 2011; González-Vega and Stein, 2012; Ji et al., 2012; Sulabo et al., 2013; Favero et al., 2014; Kong et al., 2014a; Xue et al., 2014)

[2]Means of previous studios (Opapeju et al., 2006; Lan et al., 2008; Yin et al., 2008; Woyengo et al., 2010a; Yang et al., 2010)

[3]Means of previous studies (Eklund et al., 2008; Jezierny et al., 2011; Eklund et al., 2015)

[4]Means of previous studies (Adedokun et al., 2007a; Adedokun et al., 2007c, d; Golian et al., 2008; Woyengo et al., 2010b, a; Cozannet et al., 2011; Kong and Adeola, 2013c; Toghyani et al., 2015)

[5]Means of previous studies (Adedokun et al., 2007a; Adedokun et al., 2007d; Golian et al., 2008)

[6]Means of previous studies (Adedokun et al., 2007b; Golian et al., 2008)

[7]Means of previous studies (Adedokun et al., 2007b)

[8]Means of previous studies (Adedokun et al., 2007b)

[9]Means of previous studies (Adedokun et al., 2007b)

[10]Means of previous studies (Kong and Adeola, 2013a, b)

Table 1-3 Proposed dietary composition of nitrogen-free diets for pigs and broilers, g/kg as-fed basis

Ingredients, g/kg	Pigs[1]		Broilers[2]
	Nursery	Growing-finishing	
Corn starch	544.0	790.0	200.5
Dextrose	150.0	100.0	640.0
Lactose	200.0	-	-
Vegetable oil	30.0	30.0	50.0
Cellulose	30.0	40.0	50.0
Limestone	5.0	5.0	13.0
Monocalcium phosphate	24.0	19.0	19.0
Indigestible marker	5.0	5.0	5.0
Sodium Chloride	5.0	4.0	-
Vitamin-trace mineral premix[3]	2.0	2.0	5.0
Potassium carbonate[4]	4.0	4.0	2.6
Magnesium oxide	1.0	1.0	2.0
Sodium bicarbonate	-	-	7.5
Choline chloride	-	-	2.5
Potassium chloride	-	-	2.9
Total	1000.0	1000.0	1000.0

[1]Adapted and revised from Stein et al. (2007).

[2]Adapted and revised from Adedokun et al. (2011).

[3]Vitamin and trace minerals should meet the requirement values.

[4]($Na^+ + K^+$) - Cl^- milliequivalency value is 108. Sodium requirement for 0- to 3-wk old broilers is 0.2% in NRC (1994).

Table 1-4. Determined endogenous P losses in growing pigs from previous studies

Reference	Method	Estimated EPL, mg/kg DMI	Site	Initial BW, kg	Note
Fan et al., 2001	Regression	860.0	Ileal digesta	6.8	Cannulated
	Regression	310.0	Feces	6.8	
Ajakaiye et al., 2003	Regression	118.2	Ileal digesta	40.0	Cannulated
	Regression	40.4	Feces	40.0	
Pettey et al., 2006	Regression	36.7	Feces	27.0	
	Regression	52.0	Feces	59.0	
	Regression	75.3	Feces	98.0	
Yang et al., 2007	Regression	812.0	Ileal digesta	12.5	Cannulated
	Regression	725.0	Feces	12.5	
Zhai and Adeola, 2012	Regression	882.0	Feces	15.7	
Johnston et al., 2013	Regression	250.0	Ileal digesta	31.9	Cannulated
	Regression	160.0	Feces	31.9	
Shen et al., 2002	Regression	693.0	Ileal digesta	25.0	Cannulated
	Regression	670.0	Feces	25.0	
Dilger and Adeola, 2006b	Regression	8.0	Ileal digesta	17.7	Cannulated
	Regression	2.0	Ileal digesta	17.7	
	Regression	2.5	Feces	17.7	
	Regression	6.8	Feces	17.7	
Akinmusire and Adeola, 2009	Regression	101.0	Feces	17.0	
	Regression	38.0	Feces	17.0	
	Regression	48.0	Feces	17.0	
	Regression	8.0	Feces	17.0	
Liu et al., 2016	Regression	12.9	Feces	30.9	
	Regression	-58.4	Feces	30.9	
	P-free diet	157.0	Feces	31.9	
Petersen and Stein, 2006	P-free diet	139.0	Feces	27.4	
Almeida and Stein, 2010	P-free diet	199.0	Feces	13.5	

Table 1-4. Determined endogenous P losses in growing pigs from previous studies (continued)

Reference	Method	Estimated EPL, mg/kg DMI	Site	Initial BW, kg	Note
Kim et al., 2012	P-free diet	153.0	Feces	9.2	
Almeida and Stein, 2011	P-free diet	219.0	Feces	18.8	
Rojas and Stein, 2012	P-free diet	187.0	Feces	14.0	
Almeida and Stein, 2012	P-free diet	206.0	Feces	18.2	
Son et al., 2013	P-free diet	156.4	Feces	40.0	
Almaguer et al., 2014	P-free diet	216.0	Feces	27.4	
Kim et al., 2014	P-free diet	212.0	Feces	28.3	
Jeong et al., 2015	P-free diet	168.0	Feces	55.8	
She et al., 2015	P-free diet	194.0	Feces	39.3	
	P-free diet	195.0	Feces	35.0	

Table 1-5. Determined endogenous P losses in broiler chickens from previous studies

Reference	Method	Estimated EPL, mg/kg DMI	Site	Age
Dilger and Adeola, 2006a	Regression	208.5	Prececal	15-d-old
	Regression	144.5	Prececal	15-d-old
	Regression	190.5	Excreta	15-d-old
	Regression	395.8	Excreta	15-d-old
Mutucumarana et al., 2014a	Regression	19.6	Prececal	21-d-old
	Regression	76.7	Excreta	21-d-old
	Regression	-464.1	Prececal	21-d-old
	Regression	-487.2	Excreta	21-d-old
Mutucumarana et al., 2014b	Regression	80.0	Prececal	21-d-old
	Regression	-87.0	Prececal	21-d-old
	Regression	609.0	Prececal	21-d-old
	Regression	418.0	Prececal	21-d-old
Adebiyi and Olukosi, 2015	Regression	-476.0	Prececal	14-d-old
	Regression	174.0	Prececal	14-d-old
	Regression	625.0	Excreta	14-d-old
	Regression	-201.0	Excreta	14-d-old
Mutucumarana et al., 2015a	Regression	277.0	Prececal	21-d-old
	Regression	171.0	Prececal	21-d-old
Mutucumarana et al., 2015b	Regression	49.0	Prececal	21-d-old
	Regression	142.0	Prececal	21-d-old
	Regression	-370.0	Prececal	21-d-old
Olukosi et al., 2015	Regression	1,140.0	Prececal	26-d-old
Liu et al., 2012a	Mineral-free diet	3,331.0	Excreta	24-d-old
	Mineral-free diet	4,542.0	Excreta	36-d-old
Liu et al., 2012b	P-free diet	157.0	Excreta	22-d-old

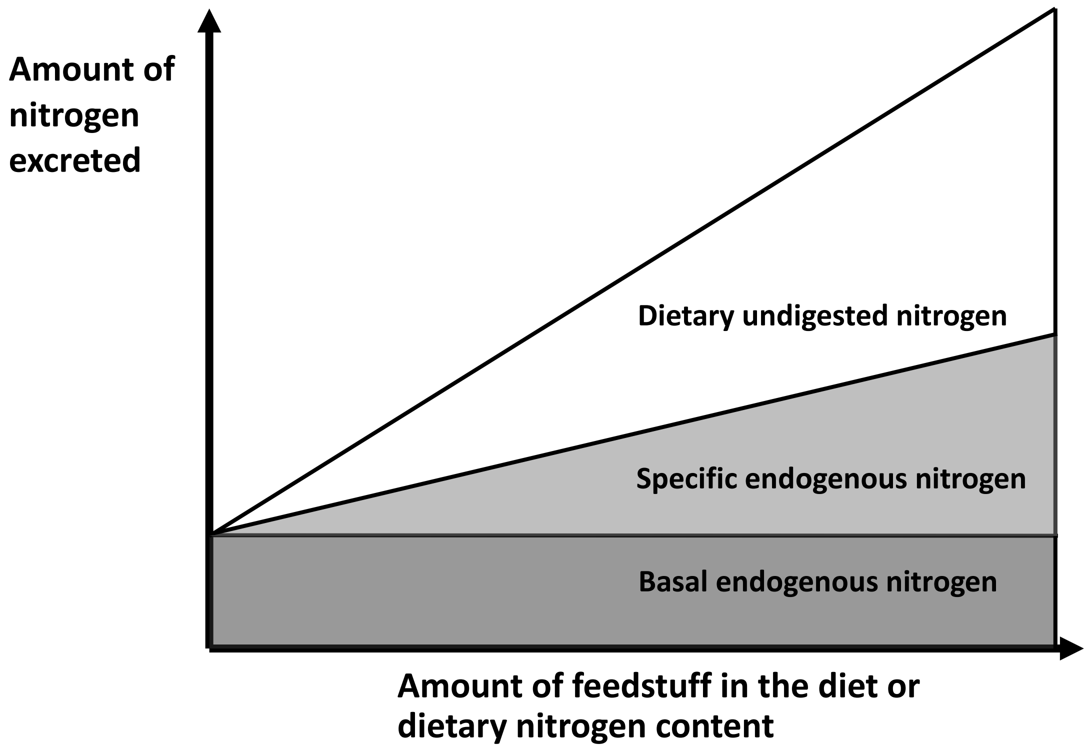

Figure 1-1 Partition of ileal nitrogen flow [adapted from McDonald et al. (2011)]

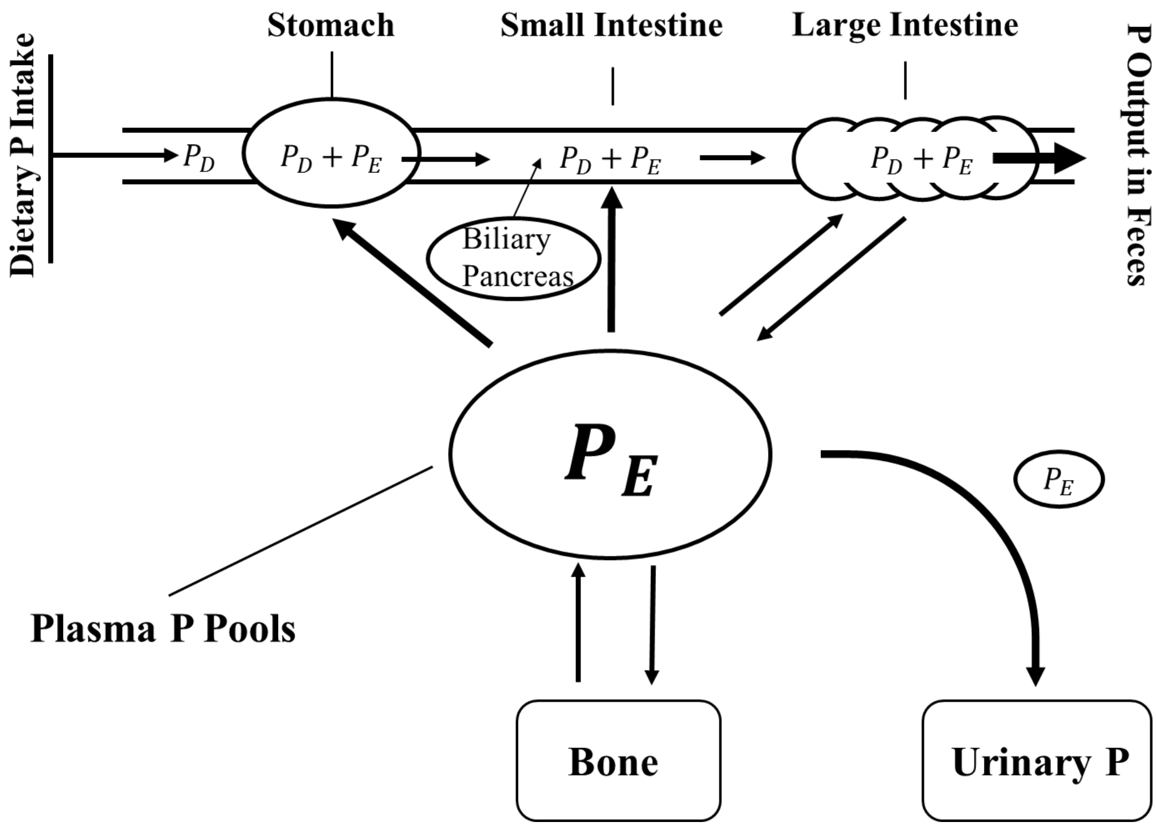

Figure 1-2. The composition of phosphorus low in the gastro-intestinal tract [adapted from Fan et al. (2001)]. P_D = dietary P; P_E = endogenous P

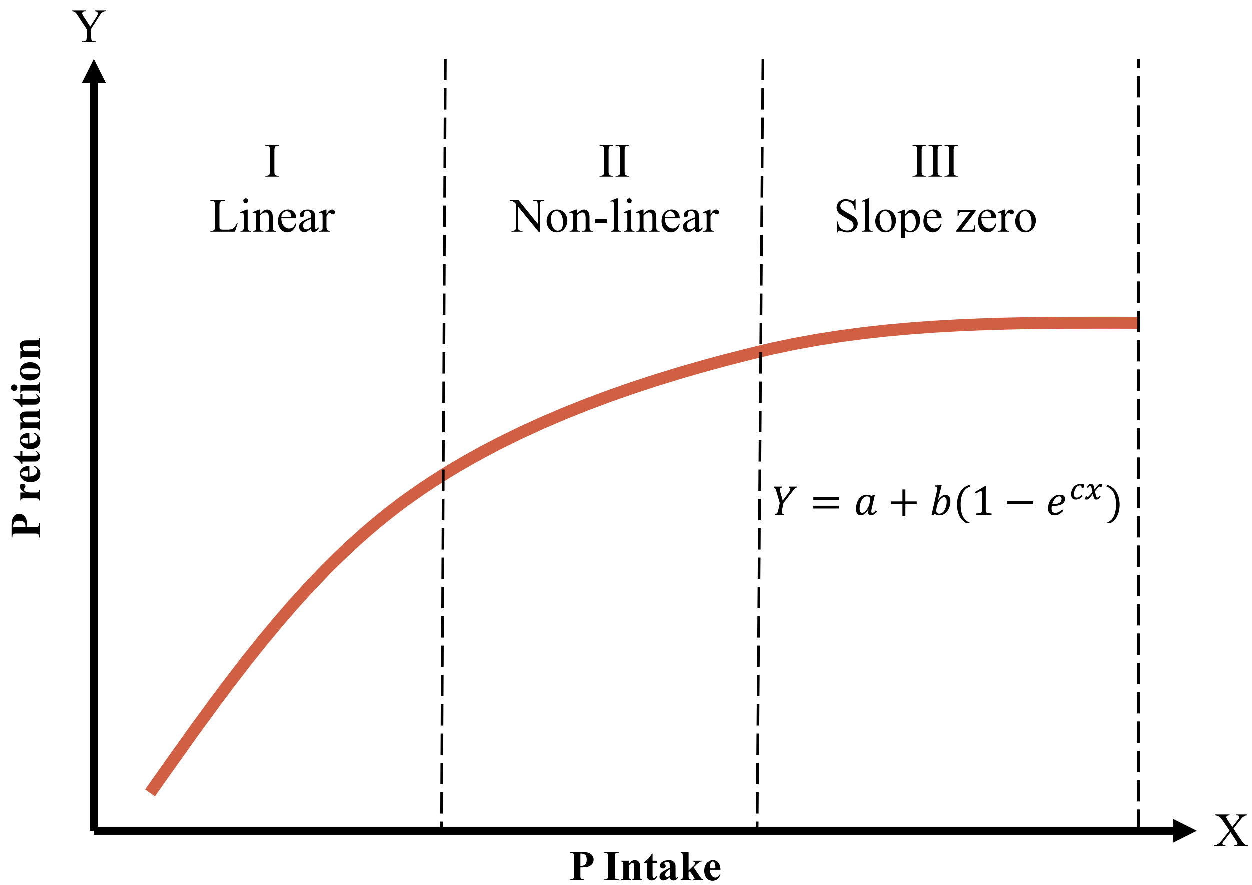

Figure 1-3. The monomolecular model of P retention. The equation is adopted from Schulin-Zeuthen et al. (2007). The response of P retention to increasing dietary P intake can be divided into three stages. I. Deficiency: the retention of P linearly increased with the P intake level; II. Marginal deficiency: diminishing response of P retention to P intake, the response might be non-linear; III: The P intake exceeds the requirement level and the slope of the regression approaches zero.

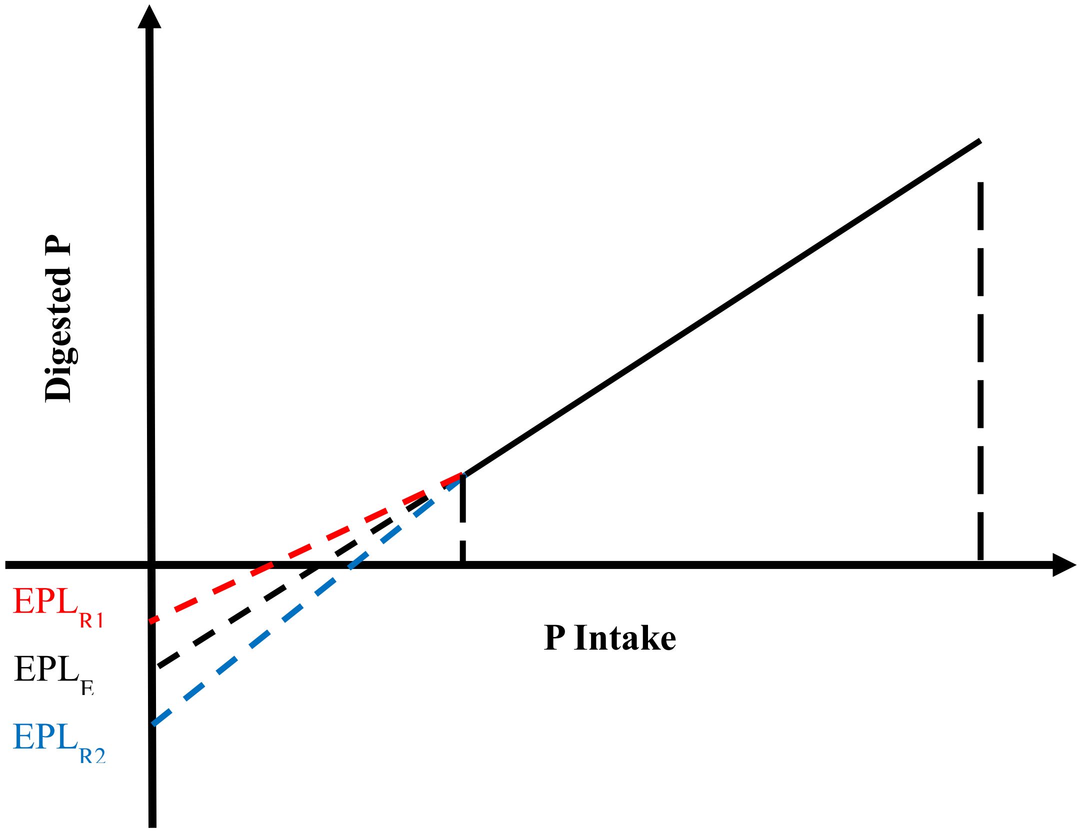

Figure 1-4. The influence of P source in the basal diet on the estimation of endogenous P losses (EPL) by using the regression method. The linear regression model is $P_D = (C_{TD} \times P_I) - EPL$. Where P_D and P_I is the amount of the digested P and P intake, respectively; C_{TD} is the coefficient of true digestibility of P. When the basal diet contains P, the intercept = $-EPL-(C_{TDC}-C_{TDB}) \times P_{IB}$. Where C_{TDC} = the true digestibility of P in the candidate ingredient; C_{TDB}= the true digestibility of P in the basal diet; P_{IB}= P intake contributed by the basal diet. The estimated EPL (EPL_E) can be the unbiased when $C_{TDC} = C_{TDB}$. The EPL_E is overestimating the EPL when $C_{TDC} > C_{TDB}$ and the real EPL is EPL_{R1}; The EPL_E is underestimating the EPL when $C_{TDC} < C_{TDB}$ and the real EPL is EPL_{R2}.

CHAPTER 2. DETERMINATION OF ADDITIVITY OF APPARENT AND STANDARDIZED ILEAL DIGESTIBILITY OF AMINO ACIDS IN DIETS CONTAINING MULTIPLE PROTEIN SOURCES FED TO GROWING PIGS

2.1 Abstract

An experiment was conducted with growing pigs to investigate the additivity of apparent (AID) or standardized (SID) ileal digestibility of CP and AA in mixed diets containing multiple protein sources. Using the determined AID or SID for CP and AA in corn, soybean meal (SBM), corn distillers' dried grains with solubles (DDGS), or canola meal (CM), the AID or SID for 4 mixed diets based on corn-SBM, corn-SBM-DDGS, corn-SBM-CM, or corn-SBM-DDGS-CM were predicted and compared with determined AID or SID, respectively. Eighteen growing pigs (initial BW = 61.3 ± 5.5 kg) were surgically fitted with T-cannulas and assigned to a duplicated 9 × 4 incomplete Latin square design with 9 diets and 4 periods. The 9 experimental diets consisted of a nitrogen-free diet (NFD) to estimate basal ileal endogenous loss (BEL) of AA; 4 semi-purified diets to determine the AID and SID of CP and AA in the 4 ingredients; and 4 mixed diets to test the additivity of AID and SID. Chromic oxide was added as an indigestible marker. Pigs were fed one of the 9 diets during each 7-d period, and ileal digesta were collected on d 6 and 7, from 0800 to 1800. The analyzed AA concentrations for the mixed diets were close to the calculated values based on the AA composition of each ingredient. The results revealed that the predicted SID were consistent with determined values, except for Leu, Thr, Asp, Cys, Pro, and Ser, in corn-SBM diet; Met and Cys in the corn-SBM-DDGS diet. The determined AID for total AA and Arg, His, Trp, Gly, and Pro in the corn-SBM diet were greater ($P < 0.05$) than predicted. For the corn-SBM-DDGS diet, the determined AID were greater ($P < 0.05$) than predicted AID for CP, total AA, and all AA except for Arg, Leu, and Pro. In the corn-SBM-CM diet, the determined AID were greater ($P < 0.05$) than predicted AID for Arg, Cys, and Gly. When compared with determined values, predicted AID in the corn-SBM-DDGS-CM diet were lower ($P < 0.05$) for total AA and Arg, Met, Cys, and Pro. In conclusion, the results substantiate the notion that SID of AA are more accurate than AID for predicting ileal digestibility of AA in mixed diets containing multiple protein sources. In addition, the lack of additivity of AID in mixed diets could be attributed to the intrinsic characteristics of the feed ingredient, especially its AA content.

Key words: additivity, amino acid, basal endogenous loss, ileal digestibility, pigs

2.2 Introduction

It is widely accepted that the nutrient requirements of livestock should be considered on a digestible nutrient basis (NRC, 2012). When diets are formulated to meet the stated requirements, the additivity of digestible nutrients is the fundamental assumption (Stein et al., 2007). It is assumed that the amount of digestible nutrients in feed is equal to the sum of that digestible nutrient from different ingredients. Thus, it is critical, when feeding, to validate the additivity assumption.

For AA, apparent (AID) and standardized (SID) ileal digestibility values have been determined for commonly used feed ingredients (Stein et al., 2007; NRC, 2012). However, it was demonstrated that AID for complete diets predicted from AID of ingredients, underestimates AID for AA and CP in diets for growing pigs (Furuya and Kaji, 1991; Stein et al., 2005). This underestimation could be a result of the relatively higher contribution of basal endogenous loss (BEL) to the total ileal AA flow for low CP ingredients such as cereal grains (Rademacher et al., 2001). For this reason, some studies suggested that SID of CP and AA in feed ingredients are more likely to be additive than AID in mixed diets because these values are independent of BEL (Furuya and Kaji, 1991; Nyachoti et al., 1997; Mosenthin et al., 2000; Stein et al., 2001, 2007). Stein et al. (2005) reported that SID for corn, soybean meal (SBM), and canola meal (CM) were additive in mixed diets based on corn-SBM, corn-CM, or SBM-CM. Thus, it is suggested that SID should be used for diet formulation (Stein et al., 2007). However, this assumption of additivity is not well established in diets containing more than 2 protein sources, which is often observed in practical swine diets. Therefore, the aim of this experiment was to investigate the additivity of AID and SID of CP and AA in mixed diets containing multiple protein sources, which are similar to practical diets for growing pigs.

2.3 Materials and Methods

The experimental protocol was approved by the Purdue University Animal Care and Use Committee (West Lafayette, IN).

2.3.1 Animals and Sample Collection

Eighteen growing pigs (initial BW = 61.3 ± 5.5 kg) were surgically fitted with T-cannulas at the ileo-cecal junction as described by Dilger et al. (2004). The pigs were assigned to 2 blocks on the basis of BW and assigned to a duplicated 9 × 4 incomplete Latin square design with 9 diets and 4 periods. All pigs were housed in two environmentally controlled rooms (ambient temperature at 21 ± 2°C) in individual floor pens with feeders and low pressure, automatic waterers under a 12-h lighting program. Pigs were fed one of the nine diets during each of the 7-d periods, and the ileal digesta were collected on d 6 and 7, from 0800 to 1800. Pigs received a daily feed allowance equivalent to 3% of the BW of the heaviest pig in each block, divided into 2 equal amounts, and fed at 0700 and 1700 h. Ileal digesta were collected into Whirlpak® bags (NASCO, Fort Atkinson, WI) containing an added 10 mL of 10% formic acid to reduce microbial activity. The bag was inspected at 30 min intervals and changed immediately as needed. Collected ileal samples were stored in a freezer at -20°C. At the end of each period, all the ileal samples from the same pig were pooled and subsampled for freeze-drying.

2.3.2 Dietary Treatments

The 9 experimental diets consisted of a nitrogen-free diet (NFD) to determine BEL of AA; 4 semi-purified diets to determine the AID and SID of CP and AA in the 4 ingredients; and 4 mixed diets to test the additivity of AID and SID (Table 2-1). The 4 ingredients were corn, SBM, corn distillers' dried grains with solubles (DDGS), and CM. The 4 mixed diets were based on corn-SBM, corn-SBM-DDGS, corn-SBM-CM, and corn-SBM-DDGS-CM. Those combinations of ingredients are common in practical diets for swine. All the mixed diets were formulated to contain approximately 16% CP, and corn starch was included as the main protein-free ingredient in the diet. Chromic oxide was added as an indigestible marker.

2.3.3 Chemical Analyses

All diets and freeze-dried ileal digesta samples were ground using a mill grinder (Retsch ZM 100, Retsch GmbH and Co., K.G., Haan, Germany) to pass through a 0.5-mm screen before analysis. The DM of diets, ingredients, and freeze-dried digesta samples were determined by drying in a force-aired oven (Precision Scientific Co., Chicago, IL) for 24 h at 105°C (Method 934.01, AOAC, 2006). Wet digestion in nitric acid and 70% perchloric acid were conducted before chromium determination (Fenton and Fenton, 1979) for the diets, ingredients, and freeze-dried digesta samples, followed by measuring absorption using a spectrophotometer at 450 nm (Spectronic 21D, Milton Roy Co., Rochester, NY). The N concentration in diets, ingredients, and freeze-dried ileal digesta samples was measured by the combustion method (Model FP2000, LECO Corp., St. Joseph, MI; [Method 990.03, AOAC, 2000]). Crude fiber (method 978.10, AOAC, 2006), acid detergent fiber [ADF; Method 973.18 (A-D), AOAC, 2006], neutral detergent fiber (NDF; van Soest et al., 1991), ash (Method 942.05, AOAC, 2006), crude fat (without acid hydrolysis) [Method 920.39 (A) AOAC, 2006] contents of the ingredients were analyzed. AA analyses [Method 982.30 E (a, b, c), AOAC, 2006] for the diets, ingredients, and freeze-dried ileal digesta samples were performed by the University of Missouri Experiment Station Chemical Laboratories (Columbia, MO).

2.3.4 Calculation and Statistical Analyses

The AID, SID, and BEL were calculated using the equations described by Dilger et al. (2004):

$$\text{AID, \%} = [1 - (Cr_i/Cr_o) \times (AA_o/AA_i)] \times 100;$$

$$BEL = AA_o \times (Cr_i/Cr_o);$$

$$\text{SID, \%} = AID + (BEL/AA_i) \times 100$$

where Cr_i and Cr_o are the concentration of chromium in diet and ileal output, respectively (mg/kg of DM); AA_i and AA_o are the concentration of AA in diet and ileal output, respectively (mg/kg of DM); and BEL (mg/kg of DMI) of an AA is calculated from the NFD. The average BEL of an AA from all pigs that received the NFD was used to derive SID. The equations were also used for calculating CP digestibility with AA replaced by N.

The predicted AID and SID of AA in mixed diets were calculated using the values determined in the semi-purified diets and the AA contributed by each ingredient to the mixed diets, using the following equation (Kong and Adeola, 2013):

$$AID_P = [(AA_C \times AID_C) + (AA_{SBM} \times AID_{SBM}) + (AA_D \times AID_D) + (AA_{CM} \times AID_{CM})] / (AA_C + AA_{SBM} + AA_D + AA_{CM}),$$

where AID_P (%) is the predicted AID for an AA in the mixed diet; AA_C, AA_{SBM}, AA_D, and AA_{CM} are the concentrations (%) of that AA contributed by corn, SBM, DDGS, and CM, respectively, which were calculated by multiplying the concentration of that AA (%) in that ingredient by the proportion (%) of the ingredient in the mixed diet; AID_C, AID_{SBM}, AID_D, and AID_{CM} are the AID (%) of the AA determined ingredient. The predicted SID of AA in mixed diets were calculated using the same equation as with AID, but SID replaced AID.

The data for AID and SID for CP and AA in each of the ingredients and mixed diets were analyzed using the MIXED procedure of SAS (9.2). The diet was considered as a fixed effect and block and period were random effects in the model. The T-test was applied to test the null hypothesis that the difference between the determined and predicted AID or SID of CP and AA for mixed diet is equal to zero. Statistical differences were established at $P \leq 0.05$; whereas $0.05 < P \leq 0.10$ was considered a trend.

2.4 Results and Discussion

The analyzed concentrations of CP, Ca, total P, crude fiber, ADF, NDF, crude fat, ash, and AA of corn, SBM, DDGS, CM are shown in Table 2-2. The analyzed CP and AA composition for experimental diets are presented in Table 2-3. The CP levels of each of the mixed diets were approximately 16%, which was close to a practical complete diet for growing pigs. The calculated N and AA concentrations in the mixed diets were similar to determined values.

The BEL data are showed in Table 2-4. The results of AID and SID for all the ingredients and mixed diets except NFD are presented in Tables 2-5 and 2-6, respectively. The AID and SID of CP and AA for corn, SBM, DDGS and CM were within the range of previous data (Bohlke et al., 2005; Baker and Stein, 2009; Woyengo et al., 2010; Yang et al. 2010; NRC, 2012). Similar to previous reports, the AID for Thr were the lowest among the indispensable AA in SBM, DDGS, and CM (72.4, 66.8, and 64.9%, respectively), and it was the second lowest in corn (67.5%). Nevertheless, the SID of Thr for ingredients were not the lowest among all essential AA. This could be attributed to the high BEL of Thr (492 mg/kg DMI). The considerable variance of BEL of Pro could be attributed to the physiological status when the pigs were fed NFD, which is a pitfall of the NFD method (de Lange et al. 1989). The BEL for most AA in the current study (Table 1-4) is similar to previous studies (Nyachoti et al., 1997; Fan and Sauer, 2002; Moter and Stein, 2004; Zhai and Adeola, 2011), whereas the ratio of endogenous loss of each AA to the loss of Lys varied from the data reported by Zhai and Adeola (2011), especially Arg (137 vs. 104), Leu (120 vs. 108), Thr (108 vs. 100), and Val (102 vs. 91).

In Table 2-7, the differences between determined and predicted values of AID and SID for CP and AA in mixed diets are presented. The results revealed that the predicted SID were consistent with determined values, except for 6 individual AA in the corn-SBM diet (Leu, Thr, Asp, Cys, Pro, and Ser) and 2 individual AA in the corn-SBM-DDGS diet (Met and Cys). In other words, the SID of CP and most AA for corn, SBM, DDGS, and CM are additive in mixed diets. These results agree with data from previous studies (Furuya and Kaji, 1991; Nyachoti et al., 1997; Mosenthin et al., 2000; Stein et al., 2001, 2005, 2007), which showed that SID of CP and AA in mixed diets could be predicted by SID of ingredients.

In the corn-SBM diet, the determined AID for total AA and 5 individual AA (Arg, His, Trp, Gly, and Pro) were greater ($P < 0.05$) than predicted (Table 2-7). For the corn-SBM-DDGS diet, the determined AID were greater ($P < 0.05$) than predicted AID for CP, total AA, and all AA except for Arg, Leu, and Pro. In the corn-SBM-CM diet, the determined AID were greater ($P < 0.05$) than predicted AID for Arg, Cys, and Gly. When compared with determined values, the predicted AID in the corn-SBM-DDGS-CM diet were lower ($P < 0.05$) for total AA and 4 individual AA (Arg, Met, Cys, and Pro). These results indicate that the predicted values derived from AID of CP and AA for ingredients were different from the determined AID in mixed diets for several AA. These results are consistent with previous data reporting that AID for AA were not additive in mixed diets containing corn for non-ruminant animals (Furuya and Kaji, 1991; Mosenthin et al., 2000; Stein et al., 2005; Kong and Adeola, 2013). However, the number of AA for which the predicted AID was different from the determined AID varied among the mixed diets used in the current study.

The addition of DDGS to the corn-SBM mixed diet to produce a corn-SBM-DDGS mixed diet resulted in the determined AID of CP and all AA but Pro being greater ($P < 0.05$) than the predicted AID (Table 2-7). An exception was Arg that showed a tendency ($P < 0.10$) for the determined AID to be greater than the predicted AID. Thus, the addition of DDGS to a corn-SBM-DDGS mixed diet resulted in more individual AA having greater determined AID than predicted AID when compared with a corn-SBM mixed diet, in which 5 individual AA were observed to have greater determined than predicted AID. However, when CM was added to the corn-SBM-based diet to produce corn-SBM-CM mixed diet, underestimation of AID was observed in only Arg, Cys, and

Gly. It could be inferred from these results that AID of AA for CM might be more likely to be additive in mixed diets than those for DDGS.

The AID of AA for high CP ingredients may be more likely to be additive in mixed diets than those for low CP ingredients. Fan and Sauer (1995) reported that the AID for CM is not underestimated if measured using the direct procedure. The study of Stein et al. (2005) supported earlier reports that AID of AA in mixed diets based on SBM and CM are additive. In contrast, the AID for low-CP ingredients might underestimate the digestibility of AA, due to the relatively higher concentration of endogenous N and AA in total ileal N and AA flow (Fan et al., 1994). Thus, if a mixed diet contains a high proportion of low-CP ingredients such as corn, the predicted AID of CP and AA are more likely to be lower than determined values, while predicted SID are similar to determined value because of the adjustment for BEL of AA. Thus, Stein et al. (2005) concluded that the additivity of AID in mixed diets depends on the N and AA content in the semi-purified diets used to determine the AID of AA in each ingredient. In diets commonly fed to pigs, the CP and AA contents are considerably higher than the semi-purified diet used to determine the AID of AA in low-protein ingredients such as corn. Therefore the AID of AA in corn tends to be underestimated, and eventually results in the lack of additivity. However, this explanation is not applicable for the DDGS used in this experiment. Although the DDGS semi-purified diet contained high concentrations of N and AA, there was still underestimation of the AID of AA in DDGS, which resulted in a lower predicted AID than determined values in corn-SBM-DDGS diet. One possible reason for this phenomenon could be the high inclusion rate of DDGS in the semi-purified diet used to determine the digestibility of CP and AA in the current study. A shortcoming of DDGS in swine diets is its high concentration of fiber (Stein and Shurson, 2009). The negative relationship between dietary fiber or non-starch polysaccharides (NSP) content and ileal digestibility of AA was reported in previous studies (Yin et al., 2000; Dilger et al., 2004). The semi-purified diet formulated with DDGS for the current study contained 57.8% DDGS. Thus, the underestimation of AID in DDGS could be attributed to the negative influence of high fiber or NSP content in the semi-purified diet that was used to determine the ileal digestibility of CP and AA in DDGS. Further studies are needed to confirm this assumption. Furthermore, the AA composition and digestibility in corn DDGS (Stein and Shurson, 2009) could lead to an unbalanced AA profile in the DDGS semi-purified diet, and therefore affect the determination of AID of AA.

It appears that ingredients could contribute to additivity or non-additivity of AID in mixed diets. In the mixed diets in the current study, SBM and CM contributed to the additivity of AID. Corn and DDGS contributed to the non-additivity of AID in mixed diets. Therefore, an explanation for the variation of differences between determined and predicted AID for the 4 mixed diets could be the different concentration of ingredients in the complete diet. Corn accounted for 37% of CP in the corn-SBM diet, while the SBM was responsible for 63% of CP. With the addition of DDGS in the corn-SBM-DDGS diet, approximately 51% of CP was derived from corn-DDGS, which contributed to the non-additivity of AID, and CP from SBM was reduced to 49%. Therefore, in the corn-SBM-DDGS diet, more AA were not additive in AID than in the corn-SBM diet. However, in the corn-SBM-CM diet the SBM-CM accounted for 68% of CP in the mixed diets. In this case, the portion of CP that originated from ingredients that contributed to the additivity of AID was greater than in the corn-SBM and the corn-SBM-DDGS diets. Consequently, there were fewer AA for which the predicted AID was different from the determined AID in the corn-SBM-CM diet. For the corn-SBM-DDGS-CM diet, the portion of CP derived from SBM-CM was lower compared with corn-SBM-CM diet, which resulted in more AA having greater determined than predicted AID.

In conclusion, the results indicate that SID of AA are more accurate than AID for predicting ileal digestibility of AA in mixed diets containing multiple protein sources. In addition, the lack of additivity of AID in mixed diets could be attributed to the intrinsic characteristics of each feed ingredient, especially its AA content.

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Table 2-1. Composition of experimental diets (% as fed)[1]

Ingredients	NFD	Corn	SBM	DDGS	CM	corn-SBM	corn-SBM-DDGS	corn-SBM-CM	corn-SBM-DDGS-CM
Corn	-	91.85	-	-	-	65.20	57.20	57.80	55.00
SBM	-	-	33.00	-	-	21.60	17.00	13.20	10.20
Corn DDGS	-	-	-	57.80	-	-	10.50	-	10.20
Canola Meal	-	-	-	-	45.00	-	-	13.20	10.00
Cornstarch	75.80	-	58.85	34.05	46.85	5.05	7.15	7.65	6.45
Dextrose	10.00	-	-	-	-	-	-	-	-
Soy oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Chromic oxide premix[2]	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Monocalcium phosphate	2.00	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Limestone	0.50	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Solka-floc	5.00	-	-	-	-	-	-	-	-
Sodium Chloride	0.40	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin premix[3]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Mineral premix[4]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Selenium premix[5]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Potassium carbonate	0.40	-	-	-	-	-	-	-	-
Magnesium oxide	0.10	-	-	-	-	-	-	-	-

[1] NFD = Nitrogen-free diet; SBM = Soybean meal; DDGS = distillers' dried grains with solubles; CM = canola meal.

[2]Provided 3.4 g Cr/kg diet.

[3]Vitamin premix supplied gram per kilogram of diet: vitamin A, 2640 IU; vitamin D_3, 264 IU; vitamin E, 17.6 IU; vitamin K activity, 2.4 mg; menadione, 880 µg; vitamin B_{12}, 15.4 µg; riboflavin, 3.52 mg; D-pantothenic acid, 8.8 mg; niacin,13.2 mg.

[4]Mineral premix supplied gram per kilogram of diet: Cu (as copper chloride), 9 mg; I (as calcium iodate), 0.36 mg; Fe (as ferrous carbonate), 194 mg; Mn (as manganese oxide), 17 mg; and Zn (as zinc oxide), 149 mg.

[5]Supplied 300 µg of Se per kilogram of diet.

Table 2-2. Analyzed nutrient composition in ingredients (% DM basis)

Items	Corn	Soybean meal	DDGS[1]	Canola meal
Crude protein	10.95	54.63	36.01	42.85
Calcium	0.01	0.31	0.02	0.72
Phosphorus	0.30	0.75	0.98	1.07
Crude fiber	1.81	2.99	7.32	11.83
Acid detergent fiber	2.5	4.5	14.5	20.4
Neutral detergent fiber	11.7	8.5	40.3	32.6
Crude fat[2]	3.3	0.7	7.5	3.1
Ash	1.5	6.9	5.0	8.0
Indispensable AA				
Arginine	0.55	4.02	1.68	2.56
Histidine	0.31	1.42	0.98	1.15
Isoleucine	0.40	2.54	1.40	1.74
Leucine	1.27	4.19	4.23	3.04
Lysine	0.39	3.51	1.23	2.38
Methionine	0.23	0.75	0.70	0.85
Phenylalanine	0.56	2.88	2.01	1.84
Threonine	0.37	2.01	1.27	1.73
Tryptophan	0.08	0.78	0.28	0.53
Valine	0.53	2.80	1.88	2.30
Dispensable AA				
Alanine	0.76	2.31	2.47	1.84
Aspartic acid	0.78	6.20	2.24	3.04
Cysteine	0.23	0.73	0.66	0.99
Glutamic acid	1.88	9.22	4.94	6.94
Glycine	0.42	2.27	1.41	2.12
Proline	0.93	2.71	2.72	2.55
Serine	0.46	2.14	1.42	1.47
Tyrosine	0.32	1.88	1.20	1.17
Total AA	10.60	52.69	33.15	38.87

[1]DDGS = distillers' dried grains with solubles.

[2]Analyzed without acid hydrolysis.

Table 2-3. Analyzed CP and AA composition of experimental diets (% as fed)[1]

Ingredients	NFD	Corn	SBM	DDGS	CM	Corn-SBM	corn-SBM-DDGS	Corn-SBM-CM	Corn-SBM-DDGS-CM
Dry matter	88.22	89.08	91.69	90.92	88.11	91.37	89.18	91.53	88.18
Crude protein	0.88	7.75	14.88	20.06	16.94	15.69	16.44	16.19	15.88
Indispensable AA									
Arginine	0.01	0.36	0.96	0.89	1.00	0.99	0.99	0.93	0.97
Histidine	0.01	0.21	0.34	0.50	0.45	0.42	0.44	0.41	0.45
Isoleucine	0.01	0.26	0.63	0.71	0.66	0.67	0.68	0.63	0.68
Leucine	0.03	0.87	1.07	2.22	1.22	1.43	1.60	1.34	1.60
Lysine	0.02	0.25	0.87	0.68	0.94	0.82	0.80	0.79	0.79
Methionine	0.00	0.15	0.18	0.37	0.33	0.24	0.27	0.26	0.31
Phenylalanine	0.02	0.36	0.68	0.99	0.72	0.80	0.84	0.73	0.83
Threonine	0.01	0.26	0.50	0.68	0.72	0.55	0.59	0.56	0.61
Tryptophan	< 0.04	0.05	0.20	0.16	0.23	0.18	0.17	0.18	0.17
Valine	0.03	0.35	0.75	0.96	0.87	0.79	0.82	0.79	0.86
Dispensable AA									
Alanine	0.02	0.52	0.60	1.33	0.75	0.82	0.93	0.79	0.95
Aspartic acid	0.02	0.52	1.52	1.22	1.21	1.51	1.48	1.30	1.35
Cysteine	0.00	0.14	0.18	0.34	0.33	0.21	0.27	0.28	0.32
Glutamic acid	0.08	1.28	2.42	2.81	2.82	2.67	2.74	2.59	2.76
Glycine	0.02	0.30	0.59	0.74	0.85	0.64	0.67	0.67	0.71
Proline	0.02	0.62	0.64	1.43	1.04	0.96	1.06	0.98	1.14
Serine	0.01	0.34	0.58	0.77	0.64	0.64	0.68	0.58	0.64
Tyrosine	0.01	0.23	0.40	0.64	0.44	0.49	0.51	0.46	0.53
Total AA	0.41	7.21	13.28	17.64	15.53	15.01	15.69	14.47	15.89

[1]NFD = Nitrogen-free diet; SBM = Soybean meal; DDGS = distillers' dried grains with solubles; CM = canola meal.

Table 2-4. Endogenous AA losses at the terminal ileum of pigs fed nitrogen-free diet[1]

	Endogenous loss, mg/kg DMI		Ratio to endogenous loss of Lys[2]		AA composition, g/16g N	
AA	Mean	SE	Mean	SE	Mean	SE
Nitrogen	2,317	249	-	-	-	-
Indispensable AA						
Arginine	541	44	137	25	3.81	0.18
Histidine	185	29	41	2	1.27	0.13
Isoleucine	331	54	71	3	2.26	0.26
Leucine	555	93	120	6	3.81	0.48
Lysine	487	106	100	-	3.26	0.45
Methionine	92	18	19	1	0.63	0.10
Phenylalanine	321	50	70	4	2.21	0.27
Threonine	492	80	108	8	3.32	0.30
Tryptophan	113	15	27	4	0.77	0.02
Valine	470	77	102	4	3.21	0.36
Dispensable AA						
Alanine	511	51	120	13	3.57	0.19
Aspartic Acid	825	139	178	8	5.60	0.60
Cysteine	186	46	38	2	1.22	0.18
Glutamic Acid	1,016	182	217	8	6.89	0.80
Glycine	1,156	154	305	72	8.07	0.80
Proline	3,171	771	923	301	22.31	4.90
Serine	445	73	99	9	3.00	0.23
Tyrosine	249	37	55	3	1.71	0.18
Total AA	11,392	1,294	-	-	-	-

[1]Means of 7 pigs. One of the pigs on the NFD did not produce enough ileal digesta for chemical analysis.

[2] Ratios are calculated by dividing endogenous loss of each AA by the endogenous loss of lysine and multiplying by 100.

Table 2-5. Apparent ileal digestibility (AID) of N and AA in ingredients and mixed diets[1], %

Items	Ingredients				Mixed Diets				SEM
	Corn	SBM	DDGS	CM	Corn-SBM	Corn-SBM-DDGS	Corn-SBM-CM	Corn-SBM-DDGS-CM	
Nitrogen	65.2	75.8	70.9	62.8	74.1	77.0	72.6	70.5	2.7
Indispensable AA, %									
Arginine	70.5	85.9	78.1	77.6	85.2	86.0	83.4	83.4	2.4
Histidine	80.1	82.6	78.0	76.2	83.5	85.4	80.6	81.2	2.1
Isoleucine	76.2	82.2	76.6	71.4	81.0	83.8	79.4	80.2	2.3
Leucine	85.5	81.1	85.9	73.8	83.0	86.7	81.2	83.1	2.3
Lysine	67.6	80.9	62.6	65.1	78.0	80.5	74.9	73.2	3.3
Methionine	86.2	82.3	84.5	82.4	83.7	88.0	84.6	86.8	2.2
Phenylalanine	80.9	81.4	73.4	70.9	80.2	83.4	78.6	77.7	2.4
Threonine	67.5	72.4	66.8	64.9	72.0	75.9	70.6	70.5	2.7
Tryptophan	60.2	83.4	71.0	76.3	80.6	81.5	79.3	77.7	2.6
Valine	73.6	79.9	74.8	68.6	78.2	81.5	76.2	77.6	2.6
Dispensable AA, %									
Alanine	78.1	72.9	80.6	68.1	76.7	81.2	75.5	77.8	2.7
Aspartic Acid	72.2	79.1	64.7	62.9	78.5	79.6	75.6	74.1	2.7
Cysteine	75.9	72.5	72.6	65.4	73.8	80.5	75.8	77.8	2.6
Glutamic Acid	83.9	84.3	82.0	79.7	85.1	86.8	84.3	84.0	1.5
Glycine	27.1	61.3	51.1	57.8	60.4	63.6	60.0	57.7	4.3
Proline	1.7	36.8	55.9	40.6	57.8	56.6	51.6	56.5	13.3
Serine	76.8	78.0	73.7	65.4	78.6	81.0	74.8	74.4	2.5
Tyrosine	78.1	79.1	80.9	69.2	80.5	83.9	78.5	80.3	2.9
Total AA	69.5	77.7	74.5	68.6	78.4	80.6	76.2	76.7	2.5

[1]Means of 8 pigs. SBM = Soybean meal; DDGS = distillers' dried grains with solubles; CM = canola meal.

Table 2-6. Standardized ileal digestibility (SID) of N and AA in ingredients and mixed diets[1], %

Items	Ingredients				Mixed Diets				SEM
	Corn	SBM	DDGS	CM	Corn-SBM	Corn-SBM-DDGS	Corn-SBM-CM	Corn-SBM-DDGS-CM	
Nitrogen	81.9	84.7	77.5	70.4	82.5	84.8	80.8	78.6	2.7
Indispensable AA, %									
Arginine	83.9	91.1	83.7	82.3	90.2	90.9	88.7	88.3	2.4
Histidine	88.0	87.6	81.4	79.8	87.6	89.1	84.7	84.8	2.1
Isoleucine	87.5	87.0	80.8	75.8	85.5	88.1	84.2	84.5	2.3
Leucine	91.2	85.9	88.1	77.9	86.6	89.7	85.0	86.1	2.3
Lysine	84.9	86.0	69.1	69.7	83.5	85.9	80.5	78.6	3.3
Methionine	91.6	87.0	86.8	84.8	87.2	91.0	87.9	89.5	2.2
Phenylalanine	84.4	81.9	82.1	74.1	82.2	85.3	80.6	81.8	2.7
Threonine	88.9	86.2	85.1	78.0	85.9	88.7	84.6	85.2	2.4
Tryptophan	80.3	88.6	77.4	80.6	86.4	87.4	85.1	83.5	2.6
Valine	85.6	85.6	79.2	73.3	83.6	86.7	81.6	82.5	2.6
Dispensable AA, %									
Alanine	86.9	80.7	84.1	74.1	82.4	86.1	81.4	82.5	2.7
Aspartic Acid	86.3	84.1	70.9	68.9	83.5	84.6	81.4	79.5	2.7
Cysteine	87.7	82.0	77.6	70.4	81.9	86.6	81.9	82.9	2.6
Glutamic Acid	90.9	88.2	85.3	82.8	88.6	90.1	87.9	87.2	1.5
Glycine	61.4	79.3	65.3	69.8	76.9	79.0	75.8	72.0	4.3
Proline	47.2	82.2	76.0	67.5	88.0	83.3	81.2	81.0	13.3
Serine	88.4	85.1	79.0	71.5	84.9	86.9	81.8	80.6	2.5
Tyrosine	87.7	84.9	84.4	74.2	85.1	88.3	83.5	84.4	2.9
Total AA	83.6	85.6	80.4	75.1	85.3	87.1	83.4	83.0	2.5

[1]Means of 8 pigs. SBM = Soybean meal; DDGS = distillers' dried grains with solubles; CM = canola meal.

Table 2-7. Differences[1] between determined and predicted values of apparent (AID) and standardized (SID) ileal digestibility (%) for nitrogen and AA in mixed diets[2]

Items	Corn-SBM		Corn-SBM-DDGS		Corn-SBM-CM		Corn-SBM-DDGS-CM	
	AID	SID	AID	SID	AID	SID	AID	SID
Nitrogen	1.7	-1.7	5.3*	2.2	4.3^{+}	1.5	1.9	-0.6
Indispensable AA, %								
Arginine	3.4**	0.8	4.8^{+}	2.3	4.2*	2.3	4.8**	2.4
Histidine	1.5*	-0.6	4.3*	2.4	0.8	-0.6	1.8	0.1
Isoleucine	0.2	-2.2	4.4**	2.1^{+}	2.0	0.3	3.1^{+}	1.0
Leucine	-0.5	-2.2*	2.6^{+}	1.2	0.1	-1.1	0.6	-0.8
Lysine	-0.4	-3.2	5.3**	2.6	1.4	-0.6	2.1	-0.5
Methionine	-0.8	-2.3	3.9**	2.5*	0.8	-0.2	2.6*	1.3
Phenylalanine	0.2	-1.8	3.6*	1.9	1.0	-0.4	1.7	0.0
Threonine	0.8	-2.9**	6.4**	2.9^{+}	1.8	-0.6	2.6	-0.3
Tryptophan	2.1*	-0.9	4.8*	2.1	2.8^{+}	0.5	3.1	0.6
Valine	0.1	-2.5^{+}	4.7*	2.3	1.7	-0.2	3.4	1.1
Dispensable AA, %								
Alanine	0.8	-1.8	4.3*	2.0	1.7	-0.1	2.1	-0.1
Aspartic Acid	0.6	-1.9**	4.1*	1.6	2.1	0.2	2.1	-0.3
Cysteine	-0.7	-3.3*	7.0**	3.6*	4.3*	1.6	6.3*	3.2
Glutamic Acid	0.5	-1.1^{+}	3.0*	1.4	1.4^{+}	0.3	1.2	-0.2
Glycine	10.9**	3.5	14.2*	7.2	10.4*	5.4	9.2^{+}	3.1
Proline	39.1**	23.7*	25.2	11.9	32.8	22.0	29.7**	17.6^{+}
Serine	0.5	-2.0*	4.3*	1.8	0.5	-1.0	0.3	-1.6
Tyrosine	1.1	-1.3	4.7**	2.6^{+}	2.4	0.6	3.1^{+}	0.9
Total AA	3.3**	0.1	5.8*	2.8	4.1^{+}	1.7	4.3*	1.5

[1]Difference is calculated by subtracting predicted AID or SID for nitrogen or individual AA from determined value; $^{+}$ means $0.05 < P \leq 0.10$; * means $P \leq 0.05$; ** means $P \leq 0.01$.

CHAPTER 3. PHOSPHORUS DIGESTIBILITY RESPONSE OF GROWING PIGS TO PHYTASE SUPPLEMENTATION OF TRITICALE DISTILLERS' DRIED GRAINS WITH SOLUBLES

3.1 Abstract

An experiment was conducted in growing pigs to determine the true total-tract digestibility (TTTD) of phosphorus (P) in triticale distillers' dried grains with solubles (triticale DDGS) with or without phytase using the regression method. Six diets were formulated in a 3 × 2 factorial arrangement, including 3 concentrations of triticale DDGS (300, 400, or 500 g/kg) and phytase (0 or 500 FTU/kg of diet). A total of 48 barrows (initial BW 22.2 ± 1.3 kg) were assigned to the 6 diets in a randomized complete block design. There was a 5-d adjustment period followed by a 5-d total collection of feces. The results show that P intake, fecal P output, and digested P increased linearly ($P < 0.01$) with increasing concentrations of DDGS in the diets. There was a main effect ($P < 0.001$) of phytase on apparent total-tract digestibility (ATTD) of P. In diets without added phytase, the ATTD of P in triticale DDGS was 65.0, 67.7, and 63.2% for the diets with 300, 400, and 500 g/kg triticale DDGS, respectively; the corresponding values for diets with added phytase were 77.3, 76.3, and 75.7%. By regressing daily digested P against daily P intake, the TTTD of P was estimated at 75.4% for triticale DDGS or 81.1% with added phytase, respectively. In conclusion, the TTTD of P in triticale DDGS without supplemental phytase was 75.4%; and 81.1% in the presence of phytase at 500 FTU/kg of

the diet, but the difference was not statistically significant. For triticale DDGS, the supplementation of 500 FTU/kg phytase in diet could increase the ATTD of P ($P < 0.001$), but not the TTTD of P.

Key words: Endogenous loss, Phosphorus, Phytase, Triticale distillers' dried grains with solubles, True digestibility

3.2 Introduction

Grains contain large amounts of P that is bound to phytate, which is poorly digested by swine. The poor digestion of phytate by swine is due largely to the limited production of endogenous intestinal phytase, an enzyme that can releases P from phytate (Adeola and Cowieson, 2011). Therefore, the supplementation of inorganic P in swine diet is necessary to meet their P requirement. Excessive P in diets will increase the P output, which may potentially cause environmental problems (Jongbloed and Kemme, 1990). In order to ameliorate the potential environmental issues, exogenous phytase addition in swine feed is common (Selle and Ravindran, 2008; Akinmusire and Adeola, 2009).

When grains are used to produce ethanol, part of the P will be released from phytate during the fermentation process. Corn contains approximately 2.6 g/kg P while about 80% of P is bound to phytate (2.1 g/kg). However, the portion of phytate P in total P is about 36% (2.6 / 7.3; NRC, 2012) in corn dried grains with solubles (DDGS), a byproduct of the biofuel industry. Although DDGS shows a higher digestibility than grain (Pedersen et al., 2007), there is still a portion of P that cannot be utilized by pigs. Therefore, phytase supplementation may improve the digestibility of P in DDGS.

The digestibility of nutrients in DDGS varies, if the DDGS are fermented from different grains (Stein et al., 2006; Urriola et al., 2009; Xue et al., 2012), making it necessary to determine the digestibility of P in DDGS produced from grains other than corn. Triticale is a hybrid of wheat and rye, which is a source of DDGS because of its high starch content (Wang et al., 1998). However, there is a dearth of information for the chemical composition and P digestibility of triticale DDGS, especially in response to dietary phytase. These data are necessary for the use of triticale DDGS in swine diet formulation. The objective of the current study was to determine the true P digestibility in triticale DDGS for growing pigs and to determine the response in P digestibility of triticale DDGS to 500 FTU/kg added phytase, using the regression method.

3.3 Materials and Methods

The experimental protocol was approved by the Purdue University Animal Care and Use Committee.

3.3.1 Animals and Sample Collection

A total of 48 Hampshire × Duroc × Yorkshire × Landrace barrows (initial BW 22.2 ± 1.3 kg) were used in the current experiment, and housed individually in stainless steel metabolism crates (0.83 by 0.71 m) equipped with a trough and nipple drinker. Randomized complete block design (RCBD) was applied in this study. The barrows were blocked by BW and randomly assigned to one of the 6 dietary treatments. The room temperature was kept at 22°C. The procedure of sample collection was the same as previously described by Zhai and Adeola (2012). There was a 5-d adjustment period followed by a 5-d total collection period with chromic oxide as a collection marker. Daily feed allowance was adjusted to approximately 3.5% of BW of the individual pig to ensure feed was completely consumed (Moter and Stein, 2004). Pigs were fed at 0700 and 1700 h during the experiment with 2 equal meals and water were supplied at 3 folds of the daily feed allowance through nipple drinkers.

3.3.2 Dietary Treatments

The chemical composition of triticale DDGS that was used in this study is in Table 3-1. To determine the true total-tract digestibility (TTTD) of P in triticale DDGS using the regression method and response to phytase, 6 diets (Table 3-2) were formulated in a 3 × 2 factorial arrangement, including 3 concentrations of triticale DDGS (300, 400, and 500 g/kg) and without or with phytase (500 FTU/kg of diet). All the P was supplied by triticale DDGS. Limestone was included to maintain a total Ca:P ratio (1.25:1) across diets.

3.3.3 Chemical Analyses

All diets and fecal samples were dried in a forced-air oven at 55°C to constant weight and then ground (Retsch ZM 100, Retsch GmbH and Co., K.G., Haan, Germany) to pass through a 0.5-mm screen before analysis. Dry matter (DM) of samples was determined by drying in a forced-air oven (Precision Scientific Co., Chicago, IL) at 105°C for 24 h (method 934.01; AOAC, 2006). Diet and fecal samples were wet digested in nitric acid and 70% perchloric acid before P determination (Fenton and Fenton, 1979). Fiske-Subbarow reducer solution and acid molybdate were added to digested samples to develop a blue color, followed by measuring absorption using a spectrophotometer at 620 nm (Spectronic 21D, Milton Roy Co., Rochester, NY). Concentration of Ca was measured using flame atomic absorption spectrometry (Varian FS240 AA Varian Inc., Palo Alto, CA, USA). Nitrogen content in the diets and ingredients was measured by the combustion method (model FP2000, LECO Corp., St. Joseph, MI; method 990.03 AOAC, 2000). Gross energy was determined in a bomb calorimeter (model 1261, Parr Instrument Co., Moline, IL). Analysis of phytic acid was performed by Eurofins Scientific Inc. (Des Moines, IA), using a modified method described by Ellis et al. (1977). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) in triticale DDGS were determined in separate samples using fiber bags and fiber analyzer (Fiber Analyzer, Ankom Technology, Macedon, NY) following a modified procedure of van Soest et al. (1991). Ether extract was determined without prior acid hydrolysis (method 934.01; AOAC, 2000).

3.3.4 Calculation and Statistical Analysis

The ATTD (%) of P and TTTD (%) of P were derived by equations described by Zhai and Adeola (2013):

$$ATTD_P = 100 \times (P_I - P_O) / P_I,$$

$$P_D = P_I - P_O,$$

$$P_D = (C_{TTTD} \times P_I) - EPL, \text{ and}$$

$$TTTD_P = C_{TTTD} \times 100,$$

Where $ATTD_P$ and $TTTD_P$ are ATTD (%) of P and TTTD (%) of P; P_I is the dietary P intake (g/d); P_O is fecal P output (g/d); P_D is digested P (g/d); C_{TTTD} is the coefficient of $TTTD_P$ estimated by regressing P_D against P_I using GLM procedure of SAS 9.2 (SAS Inst. Inc., Cary, NC); EPL is endogenous P loss (g/d).

The P intake, P output, digested P, and ATTD of P data were analyzed using the MIXED procedure of SAS 9.2. Individual pig was considered the experimental unit for all statistical analyses. Statistical significance was determined at $P < 0.05$. Levels of triticale DDGS (300, 400, or 500 g/kg), the phytase supplementation (0 or 500 FTU/kg) and the interaction between levels of triticale DDGS and phytase supplementation were included in the analysis model, block was considered as random effect. Contrasts were used to examine linear and quadratic effects of increasing triticale DDGS concentrations without or with added phytase. Analysis of covariance (ANCOV) using proc GLM of

SAS with solution option was used to estimate the slopes (estimates of TTTD of P) and intercepts (estimates of endogenous loss of P) of the two linear regression lines. The statistical model was P_D = phytase P_I phytase*P_I, where P_D is as defined above; phytase was coded as a dummy variable (0 or 1 for without or with added phytase, respectively) and included in the class statement; P_I is as defined above and was not included in the class statement; and phytase*P_I is the interaction of phytase and P_I. Within this model, phytase tested the difference between the intercepts for 0 and 500 FTU/kg, phytase*P_I tested the difference between the slopes.

3.4 Results

The levels of DM, CP, GE, ether extract (EE), ADF, NDF, and phytic acid are presented in Table 3-1. Analyzed phytase activity were 516, 936, and 890 FTU/kg in diets formulated to contain 300, 400, and 500 g DDGS/kg with added phytase, respectively. The difference in phytase activity could be due to analytical variation. In diets without added phytase the analyzed phytase activity was close to zero (Table 3-2, footnote 5). The results of analyzed values of nitrogen, GE, Ca, and P of the experimental diets were close to the calculated nutrient composition in Table 3-2.

There were linear increases ($P < 0.05$) in P intake, total tract P output, and total tract digested P as triticale DDGS concentrations increased, without or with added phytase (Table 3-3). Quadratic effect ($P < 0.05$) was observed only in total tract digested P with increasing triticale DDGS, without added phytase. In diets without added phytase, the ATTD of P in triticale DDGS were determined to be 65.0, 67.7, and 63.2% for the diets containing 300, 400, and 500 g/kg triticale DDGS, respectively; the corresponding values for diets with added phytase were 77.3, 76.3, and 75.7%. There was a main effect

(*P* < 0.001) of phytase on improving ATTD of P in triticale DDGS. Blocks, concentrations of DDGS, and the interaction between phytase and concentrations of DDGS did not affect ATTD of P.

The regression of daily digested P against daily P intake is presented in Figure 3-1. The estimated EPL was 0.172 and 0.080 g/d for diets without or with added phytase. The intercepts of the regression for 0 and 500 FTU/kg were neither different from each other nor from zero. The TTTD of P was estimated to be 75.4 or 81.1% for triticale DDGS without or with added phytase, respectively. The slopes were not different from each other. Therefore, adding 500 FTU/kg phytase did not increase TTTD of P in triticale DDGS in the current study.

3.5 Discussion

The chemical composition of triticale DDGS used in the current study was similar to that reported in the NRC (2012) for CP and P. In previous studies, the concentrations of CP in triticale DDGS varied from approximately 27.8% to 36.7%. Values of EE, ADF, and NDF in triticale DDGS also varied between samples used in different studies (McKeown et al., 2010; Oba et al., 2010; Oryschak et al., 2010; Wierenga et al., 2010). Previous studies combined with the current experiment show that the nutrient composition in triticale DDGS varies, especially in the concentration of CP. It has been shown that the portion of P in phytic acid is 28.2% (Selle et al., 2009). In the current study, the concentration of phytic acid in triticale DDGS was 8.6 g/kg (DM basis). Therefore, the content of phytate P was 2.4 g/kg (DM basis). The total P concentration was 6.9 g/kg (DM basis) in the triticale DDGS used in current study. Thus, the portion of phytate-bound P in total P was 35.4% (2.4 / 6.9). The concentration of phytate-bound P and the portion of that in total P are similar to corn and wheat DDGS (Yáñez et al., 2011; Almeida and Stein, 2012; NRC, 2012).

It is widely accepted that P in DDGS is highly available, because some of the P bound to the phytate complex in grain is hydrolyzed during the fermentation process (Pedersen et al., 2007). In the current study, the average ATTD of P for the 3 triticale DDGS concentrations without added phytase was 65%, which translates to a 35% P indigestibility. This is consistent with the proportion of phytate-bound P in total P in the triticale DDGS at 35.4% used in the current study. There is a lack of data focusing on the availability of nutrients from triticale DDGS in diets for swine. Compared with DDGS from other sources, triticale DDGS in the current study has a similar ATTD value of P with corn DDGS (Stein and Shurson, 2009) and wheat DDGS (NRC, 2012). With the supplementation of 500 FTU/kg phytase, the average ATTD of P in triticale DDGS increased from 65.3% to 76.4%. Thus, the addition of phytase in diets could increase the ATTD of P in triticale DDGS. Furthermore, it was shown in Table 3-3 that in the three diets without additional phytase, the increase of digested P was in a quadratic patterns, while in diets contained 500 FTU/kg supplemental phytase the pattern was just linear. This phenomenon suggested that there was a limitation of P digestion when high concentrations of DDGS was included in diet, and the addition of phytase ameliorated this negative effect. Actually, based on previous studies, the beneficial effect of phytase on P digestibility in DDGS is not as consistent as in grains like corn (Xu et al., 2006; Almeida and Stein, 2010; Yáñez et al., 2011), which could be due to the partial release of phytate-bound P in the grain during fermentation in the production of DDGS.

In the current study, the TTTD of P in triticale DDGS was estimated at 75.4 and 81.1% without or with additional phytase in the triticale DDGS, by the regression method. The application of the regression method on estimating digestibility of nutrient is well documented in previous studies (Fan and Sauer, 1997; Fan et al., 2001; Dilger and Adeola, 2006). In this experiment, the TTTD of P in triticale DDGS without added phytase (75.4%) is higher than the value of corn and wheat DDGS (65 and 61%, respectively), summarized in NRC (2012).

The estimated EPL in this study were 0.172 and 0.080 g/d for groups without and with added phytase, respectively, but these values are not different from zero (Figure 3-1). Average daily feed intakes were 0.621 or 0.628 kg for pigs on diets without or with added phytase, respectively. Thus, estimated EPL were 277 and 127 mg/kg DMI. The NRC (2012) EPL of 190 mg/kg DMI was generated from pigs fed P-free diets. There are intrinsic differences between the regression method and P-free diet method. The distance between the lowest P concentration and zero P intake intercept in the current study is prone to large variation. These results suggested that when the regression method was applied to estimate the endogenous loss of P, the concentrations of P in experimental diets should be considered.

Phytase addition at 500 FTU/kg numerically increased the TTTD of P in triticale DDGS from 75.4 to 81.1%, but the increase was not statistically significant. However, as indicated above, phytase supplementation at 500 FTU/kg significantly increased the ATTD of P in triticale DDGS from 65.3% to 76.4%. The inconsistent response between the ATTD and TTTD of P to phytase may be due to several possible reasons. The numerical difference in the EPL for the group without added phytase at 0.172 g/d and

0.080 g/d for the group with added phytase implies that phytase numerically decreased endogenous P loss. Although the two estimates of EPL were neither significantly different from each other nor from zero, the numerical reduction in endogenous P loss partially explains the smaller difference between TTTD of P at 75.4% for no added phytase vs. 81.1% for added phytase compared with average ATTD of P at 65.3% for no added phytase vs. 76.4% for added phytase. Considering the definition of ATTD and TTTD, the change in EPL would affect the estimate of TTTD. Thus, a numerically reduced EPL, the part that was added back to the ATTD to derive TTTD, was also smaller in groups with added phytase. As a result, the observed difference between the TTTD of P for the groups without and with added phytase was smaller compared with that for ATTD. In the current study, the added 500 FTU/kg phytase increased the ATTD of P by approximately 11-percentage points from 65.3 % to 76.4%, which was statistically significant. However, the increase in TTTD of P with phytase supplementation was less than 6-percentage points from 75.4 % to 81.1%, which was not statistically detectable.

In conclusion, the TTTD of P in triticale DDGS without supplemental phytase was determined to be 75.4%; and 81.1% in the presence of added phytase at 500 FTU/kg of the diet but the difference was not statistically significant. For triticale DDGS, phytase supplementation at 500 FTU/kg of diet increased the ATTD of P, but not the TTTD of P.

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Table 3-1. Chemical composition of the triticale distillers' dried grains with solubles (Triticale DDGS) used in this study (DM basis)

Item, g/kg	Triticale DDGS
Dry matter	927
Nitrogen	44.4
Energy, kcal/kg	5,298
Ether extract	56.4
ADF[1]	153.5
NDF[2]	357.2
Ca	0.9
P	6.9
Phytic acid[3]	8.6

[1] ADF = acid detergent fiber.

[2] NDF = neutral detergent fiber.

[3]Analysis of phytic acid was performed by Eurofins Scientific Inc. Method reference: Ellis et al. (1977) (modified).

Table 3-2. Ingredients and nutrient composition of the experimental diets (DM basis)

[1]Triticale DDGS = Triticale distillers' dried grains with solubles

[2]Vitamin premix supplied per kg of diet: vitamin A, 2,423 IU; vitamin D_3, 242 IU; vitamin E, 17.6 IU; vitamin K activity, 2.4 mg; menadione, 804 µg; vitamin B_{12}, 14.1 µg; riboflavin, 2.8 mg; d-pantothenic acid, 9 mg; and niacin, 13 mg.

Ingredients, g/kg	Triticale DDGS[1] without added phytase			Triticale DDGS with added phytase		
Triticale DDGS[1]	300	400	500	300	400	500
Cornstarch	467.7	365.7	263.7	457.7	355.7	253.7
Sucrose	200	200	200	200	200	200
Soy oil	20	20	20	20	20	20
Sodium Chloride	3.3	3.3	3.3	3.3	3.3	3.3
Limestone,	6	8	10	6	8	10
Vitamin premix[2]	1.5	1.5	1.5	1.5	1.5	1.5
Mineral premix[3]	1	1	1	1	1	1
Se premix[4]	0.5	0.5	0.5	0.5	0.5	0.5
Phytase premix[5]	0	0	0	10	10	10
Total	1000	1000	1000	1000	1000	1000
Analyzed composition						
P, g/kg	2.23	2.96	3.43	2.36	2.89	3.60
Ca, g/kg	2.77	3.66	4.37	2.99	3.61	4.55
Ca:P	1.24	1.24	1.27	1.27	1.25	1.26
Calculated composition						
Gross Energy, kcal/kg	4233	4318	4402	4193	4278	4363
Crude Protein, g/kg	77	103	129	77	103	129
Total amino acids, g/kg						
Arginine	4.3	5.8	7.2	4.3	5.8	7.2
Histidine	2.0	2.7	3.4	2.0	2.7	3.4
Isoleucine	3.7	4.9	6.1	3.7	4.9	6.1
Leucine	6.7	8.9	11.1	6.7	8.9	11.1
Lysine	2.3	3.0	3.8	2.3	3.0	3.8
Methionine	1.6	2.1	2.6	1.6	2.1	2.6
Phenylalanine	4.7	6.3	7.9	4.7	6.3	7.9
Threonine	3.2	4.2	5.3	3.2	4.2	5.3
Tryptophan	2.7	3.6	4.6	2.7	3.6	4.6
Valine	4.6	6.2	7.7	4.6	6.2	7.7

[3]Mineral premix supplied per kg of diet: Cu (as copper sulfate), 9 mg; I (as calcium iodate), 0.34 mg; Fe (as ferrous sulfate), 97 mg; Mn (as manganese oxide), 12 mg; and Zn (as zinc oxide), 97 mg.

[4]Supplied 300 μg of Se per kg of diet.

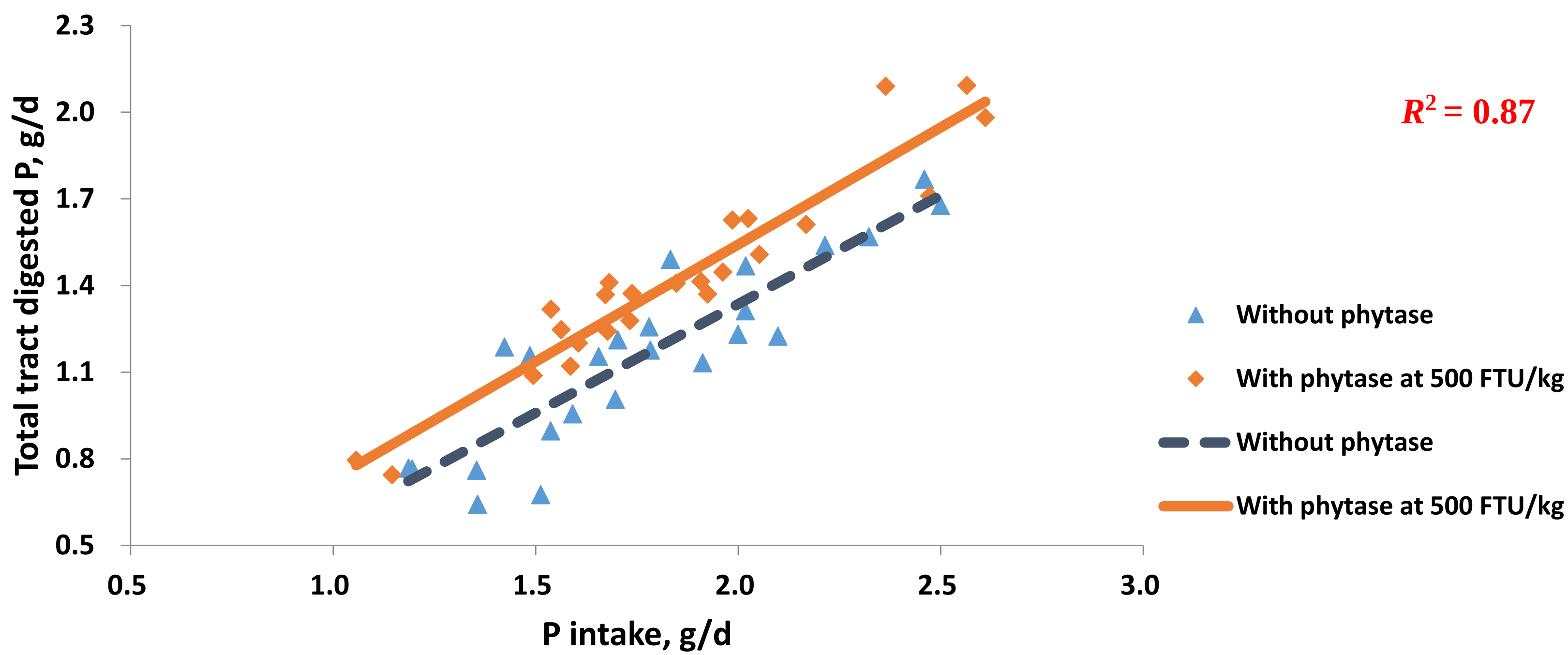
[5]Added to provide 500 FTU/kg phytase in complete diet. The analyzed phytase activity was 516, 936, and 890 FTU/kg in diets containing 300, 400 and 500 g/kg DDGS with added phytase. In diets without added phytase the analyzed phytase activity was 64, 73, and 17 FTU/kg in diets containing 300, 400 and 500 g/kg DDGS.

Table 3-3. Dietary P intake, total tract P output and apparent P digestibility of growing pigs fed diets containing different concentrations (g/kg) of triticale distillers' dried grains with solubles (triticale DDGS) without or with phytase[1]

Item	Triticale DDGS without added phytase			Triticale DDGS with added phytase[1]			Pooled SEM	*P* value without added phytase		*P* value added phytase	
	300	400	500	300	400	500		L	Q	L	Q
P intake, g/d	1.424	1.873	2.032	1.456	1.861	2.200	0.069	< 0.001	0.103	< 0.001	0.725
Total tract P output, g/d	0.497	0.605	0.725	0.321	0.437	0.526	0.045	0.005	0.914	0.017	0.833
Total tract digested P, g/d	0.927	1.268	1.307	1.135	1.424	1.673	0.045	0.004	0.034	< 0.001	0.757
Apparent total tract digestibility[2], %	65.0	67.7	63.2	77.3	76.3	75.7	2.2	0.262	0.205	0.700	0.942
n	8	8	8	8	8	8					

[1]Added to provide 500 FTU/kg phytase in complete diet.

[2]Main effect of Phytase on ATTD of P at $P < 0.001$



Item	Intercept	SE for intercept	Slope	SE for slope	Estimated endogenous P loss, g/d	Estimated total tract P digestibility, %
Without phytase	**0.172**	**0.130**	**0.754**	**0.072**	**0.172**	**75.4**
With phytase at 500 FTU/kg	**0.080**	**0.127**	**0.811**	**0.067**	**0.080**	**81.1**

Figure 3-1. Regression of digested P against P intake in different levels of triticale DDGS, without or with added phytase. Total P digested (g/d) was regressed against dietary P intake (g/d), for diets without or with added phytase.

The statistical model was P_D = phytase P_I phytase*P_I, where P_D is digested P (g/d); phytase was coded as a dummy variable (0 or 1 for without or with added phytase, respectively) and included in the class statement; P_I is dietary P intake (g/d) and was not included in the class statement; and phytase*P_I is the interaction of phytase and P_I. The regression intercept provides an estimate of endogenous P loss (g/d), the slope represents true total tract P digestibility. The *P* values for the intercepts were 0.19 and 0.53 (compared to zero) for the regression lines without and with added phytase, respectively. The *P* values for the slopes were both < 0.001 (compared to zero) for the regression lines without and with added phytase, respectively. The intercepts of the regression for 0 and 500 FTU/kg were neither different from each other nor from zero. The slopes were not different from each other. The overall *P* value for the regression model was < 0.001.

CHAPTER 4. DIETARY NITROGEN CONCENTRATION AFFECTS ILEAL PHOSPHORUS DIGESTION IN GROWING PIGS

4.1 Abstract

A study was conducted to investigate the effect of dietary CP concentrations on ileal phosphorus (P) digestion in growing pigs. A total of 18 ileal-cannulated pigs (initial BW 44.2 ± 3.2 kg) were used in a duplicated 9 × 3 incomplete Latin Square design, with 9 treatments and three 7-d experimental periods giving 6 replicates per treatment. The 9 treatments consisted of one nitrogen-free diet to estimate basal endogenous loss of AA, and 8 corn-soybean meal-based diets in a 2 × 4 factorial arrangement, which included 2 CP concentrations (6.9 or 13.4%) and 4 apparent total tract digestible P (ATTDP) concentrations (0.09, 0.16, 0.24, or 0.32%). Soybean meal and mono-calcium phosphate were used to adjust the CP concentrations and ATTDP concentrations, respectively. Limestone was included in diets to maintain a Ca: ATTDP ratio of 2.5 across treatments. Ileal digesta was collected continuously for 10 h on the last 2 d of each 7-d experimental period. The ileal digesta samples were lyophilized and analyzed to calculate ileal digested N and P. Data was analyzed using the PROC MIXED procedure of SAS (9.3) and contrasts were used to test the linear and quadratic effects of increasing levels of P within each CP concentration. In the low CP groups (6.9%), the ileal digested P were 0.71, 1.16, 1.64, and 2.03 g/kg•DMI for diets that contained 0.09, 0.16, 0.24, and 0.32% ATTDP, and were 0.70, 1.54, 2.03, and 1.99 g/kg•DMI for their counterparts in the high CP group (13.4%). There was a main effect of CP level ($P < 0.05$) on ileal digested P (g/kg•DMI). The ileal digested P (g/kg•DMI) increased linearly ($P < 0.01$) with increasing ATTDP concentrations in the low CP group, but the pattern was linear ($P < 0.01$) and quadratic ($P < 0.01$) in the high CP group. In the low and high CP diets, the determined true ileal digestibility of P in mono-calcium phosphate was 54.4% and 75.6%, respectively. In conclusion, this research indicated that the ileal digestion of P could be limited by protein deficiency. The dietary CP concentration should be considered in P digestibility related studies.

Key words: amino acids, ileal digestibility, phosphorus, pigs

4.2 Introduction

For swine, amino acids (AA) and phosphorus (P) are both essential nutrients playing roles in numerous biochemical reactions, such as protein synthesis and ATP production. The use of AA and P in feed is important in swine nutrition; for this reason, there are many studies published which aim to determine the digestible content of AA and P in feed ingredients (Stein et al., 2007; Zhai and Adeola, 2012; Kim et al., 2014) and the factors affecting AA and P digestion (Johnston et al., 2004; Zhai and Adeola, 2011). However, in those studies, AA and P digestion have been considered separately. In studies determining P digestibility in feed ingredients, the P concentration and Ca:P ratio in experimental diets are typically adjusted because those factors can impact the result of P digestibility (Koshihara et al., 2004). However, crude protein (CP) concentration in diets is usually not considered as a factor influencing P digestion, because it is assumed that dietary CP concentration does not affect P digestion.

In studies focused on the determination of P digestibility in high protein content feed ingredients such as SBM and DDGS, the dietary CP concentration in experimental diets usually can be maintained at a concentration close to practical diets (Dilger and Adeola, 2006; Xue and Adeola, 2015). For studies on feed ingredients which have very low CP content, like mono-calcium phosphate, the dietary CP in the basal diet has also been adjusted to a level similar to commercial pig diets (Zhai and Adeola, 2011). There is a lack of studies evaluating the effect of dietary CP concentration on the P digestion in growing pigs. Likewise in AA digestibility studies, diets are typically maintained at similar CP concentrations as this may influence AA and N digestibility (Zhai and Adeola, 2011; Xue et al., 2014). In these studies, the P concentration is not usually adjusted across experimental diets. The assumption that CP and P absorption are independent has not been well established and the relationship between protein and P retention could be more complicated than previously assumed.

In fact, there is a strong correlation between the whole body content of N and P in growing pigs (NRC, 2012). These data suggest that the amount of deposited N and P are correlated with each other. These two nutrients could be linked in various aspects. For example, the lean tissue growth of pigs relies upon protein synthesis and bone development, where AA and P are both key regulators and components (Kimball et al., 2002; Frank et al., 2005; Escobar et al., 2006). In addition, AA and P could interact with each other even from the digestion phase. The active transportation of P in small intestine is dependent on the sodium-phosphate co-transporter IIb (NaPi-IIb), which is a protein synthesized in epithelial cells (Adedokun et al., 2012). Considering the roles of protein and P in tissue growth and biochemical reactions, it is reasonable to hypothesize that there might be a link between AA and P absorption and deposition in pigs. Therefore, dietary CP concentration could affect the digestion of P, or dietary P concentration could have an effect on CP digestion. The relationship between AA and P absorption in pigs is still not clearly defined, hence in this study we determined if different dietary CP concentrations can affect the response of digestion of P in growing pigs to varying dietary P concentrations.

4.3 Materials and Methods

The experimental protocol used in this study was approved by the Purdue University Animal Care and Use Committee.

4.3.1 Animals and Sample Collection

A total of 18 barrows (initial BW = 44.2 ± 3.2 kg) were surgically fitted with ileal-cannulas as described by (Dilger et al., 2004) and assigned to a duplicated 9 × 3 incomplete Latin Square design, with 9 experimental diets and 3 periods. The pigs were blocked by BW, and the daily feed allowance was equivalent to 3% of the BW of the heaviest pig in each block, divided into 2 equal amounts, and fed at 0700 and 1700 h. The pigs were all housed in individual floor pens with low pressure, automatic waterers and feeders in two environmentally controlled rooms (ambient temperature 21 ± 2°C). During each of the three 7-d periods, ileal digesta were collected continuously on d 6 and 7, from 0800 to 1800 into Whirlpak® bags (NASCO, Fort Atkinson, WI) containing 10 mL of 10% formic acid to reduce microbial activity. The bag was checked at 30 min intervals and changed immediately as needed. Collected ileal samples were frozen at -20°C. At the end of each period, all the ileal samples from the same pig were pooled and subsampled for lyophilization.

4.3.2 Dietary Treatments

The 9 treatments consisted of 1 nitrogen free diet to estimate basal endogenous loss of AA, and 8 corn-soybean meal (SBM) based diets in a 2 × 4 factorial arrangement (Table 4-1), which included 2 CP concentrations (6.9 or 13.4%, equal to 50 and 100% of requirement, respectively), and 4 apparent total tract digestible P (ATTDP) concentrations (0.09, 0.16, 0.24, or 0.32%, equal to 40, 70, 100, and 130% of requirement, respectively). For the convenience of expression, the four concentrations of ATTDP were noted as low, mid-low, mid-high, and high in this manuscript. Through these treatments we could determine if CP deficiency would affect the pattern of P absorption in small intestine. Mono-calcium phosphate was used to adjust the ATTDP concentration. Therefore, the regression method can be applied to determine the true ileal digestibility of P in mono-calcium phosphate within low and high CP diets, respectively. Limestone was included in diets to maintain a Ca: ATTDP ratio of 2.5 across treatments. Chromic oxide was added in diets as an indigestible marker.

4.3.3 Chemical Analyses

All diets and lyophilized ileal digesta samples were ground using a mill grinder (Retsch ZM 100, Retsch GmbH and Co., K.G., Haan, Germany) to pass through a 0.5-mm screen before analysis. The DM of diets and digesta samples were determined by drying in a forced-air oven (Precision Scientific Co., Chicago, IL) for 24 h at 105°C (Method 934.01, ((AOAC), 2006). The N concentration in the diets and ileal digesta samples was measured by the combustion method (Model FP2000, LECO Corp., St. Joseph, MI; AOAC, 2000). The analysis of diets and ileal digesta for AA [Method 982.30 E (a, b, c), AOAC, 2006] were conducted by the University of Missouri Experiment Station Chemical Laboratories (Columbia, MO). Chromium and P concentration was analyzed after wet digestion in nitric acid and 70% perchloric acid, as described by Fenton and Fenton (1979), followed by measuring absorption using a spectrophotometer at 450 nm for chromium determination (Spectronic 21D, Milton Roy Co., Rochester, NY). Fiske-Subbarow reducer solution and acid molybdate were added to digested samples to develop a blue color, followed by measuring absorption using a spectrophotometer at 620 nm for P determination (Spectronic 21D, Milton Roy Co., Rochester, NY).

4.3.4 Calculation and Statistical Analysis

The apparent ileal digestibility (AID), basal endogenous loss (BEL) of AA, and standardized ileal digestibility (SID) of N and AA were calculated using the equations described by Dilger et al. (2004):

$$AID, \% = [1 - (Cr_i/Cr_o) \times (AA_o/AA_i)] \times 100;$$

$$BEL = AA_o \times (Cr_i/Cr_o);$$

$$SID, \% = AID + (BEL/AA_i) \times 100;$$

where Cr_i and Cr_o are chromium concentration in diet and ileal output, respectively (mg/kg of DM); AA_i and AA_o are the AA concentration in diets and ileal digesta samples, respectively (mg/kg of DM). The equations were also used for calculating CP digestibility with AA replaced by N. The average BEL (mg/kg of DMI) of N and AA from all pigs that received the nitrogen-free diet (NFD) was used to the derive SID. P digestibility was calculate using the same equation as AID of AA by replacing AA with P. The digested P (g/kg DMI) was calculated based on the P concentration in the diet and AID of P.

The regression method was applied to estimate the true ileal digestibility of P in mono-calcium phosphate. Due to the possibility that the excessive dietary P concentration in the high ATTDP groups may limit the efficiency of P absorption and utilization, only the ileal digested P (g/kg DMI) in the low, med-low, and med-high ATTDP groups was regressed against dietary P intake (g/kg DMI), for diets with low or high CP concentration. The statistical model in PROC GLM of SAS (9.3) was:

$$P_D = Level_{CP} \quad P_I \quad Level_{CP} *P_I,$$

where P_D is ileal digested P (g/kg DMI); $Level_{CP}$ was coded as a dummy variable (0 for low CP diets; 1 for high CP diets) and considered as class variable; P_I is dietary P intake (g/kg DMI) and was not included in the class statement; and $Level_{CP}$ *P_I is the interaction of $Level_{CP}$ and P_I. The regression slope represented an estimation of true ileal P digestibility of mono-calcium phosphate.

The rest of the data was analyzed by using the PROC MIXED procedure of SAS (9.3) and contrasts were used to test the linear and quadratic effects of increasing concentrations of P within each CP concentration. Statistical differences were established at $P < 0.05$.

4.4 Results

The diets in this study were formulated on the basis of ATTDP concentrations, which resulted in the total P concentration across low and high CP group being inequivalent. For this reason, the comparison of digestibility would be biased in the current study; therefore, the results of P digestion will be reported and discussed on a g/kg DMI basis.

The results of ileal N and P digestion are shown in Table 4-2. In the low CP group (6.9%), the ileal digested P were 0.71, 1.16, 1.64, and 2.03 g/kg DMI for diets containing low, mid-low, mid-high, and high concentration of ATTDP, and their counterparts in the high CP group (13.4%) were 0.70, 1.54, 2.03, and 1.99 g/kg DMI. Low dietary CP concentration decreased ($P < 0.05$) ileal digested P (g/kg DMI). This effect was clear in mid-low and mid-high ATTDP diets (1.16 vs. 1.54 and 1.64 vs. 2.03, respectively). Meanwhile, the dietary P concentrations that were needed for maximum P absorption were different in the two CP groups. The ileal digested P (g/kg DMI) increased linearly ($P < 0.01$) with increasing ATTDP concentrations in the low CP group, but the pattern was linear ($P < 0.01$) and quadratic ($P < 0.01$) in the high CP group. In NFD group, the P digestion was determined to be 1.17 g/kg DMI.

The results of linear regression are presented in Figure 4-1. The slopes represented the estimated true ileal digestibility of P in mono-calcium phosphate. The overall P value for the regression model was < 0.001, $R^2 = 0.788$. In the low CP group, the linear regression equation was Y = 0.544 (SE = 0.087, $P < 0.001$) X – 0.357 (SE = 0.271, $P = 0.199$). The linear regression equation for the high CP group was Y = 0.756 (SE = 0.088, $P < 0.001$) X – 1.412 (SE = 0.343, $P < 0.001$). The slopes were not statistically different from each other ($P = 0.098$).

As shown in Table 4-2, there was a main effect of dietary ATTDP concentrations ($P < 0.05$) on apparent and standardized ileal digested N (g/kg DMI). In the high CP group, the standardized ileal digested N were 20.99, 23.49, 22.37, and 21.90 g/kg DMI, for diets containing low, mid-low, mid-high, and high concentrations of ATTDP, respectively. This result showed a quadratic effect of dietary P concentration on CP digestion ($P < 0.01$).

The result of AID of CP and AA are shown in Table 4-3. The main effect of dietary CP was observed in AID of N and all the individual AA ($P < 0.01$), where the AID determined in the low CP group were lower compared with the high CP group. The effect of dietary P on AID for N and AA was not observed in the current study. The results of BEL of AA are shown in Table 4-4. In Table 4-5, the results of SID for N and AA are presented. The effect of dietary CP on SID was observed in all the N and individual AA ($P < 0.05$) except for Proline. The SID of N and AA determined in the high CP diets were greater than that in the low CP group ($P < 0.05$), except for Proline.

There was no effect of P and the interaction between dietary N and P concentrations on SID of N and AA in this study.

4.5 Discussion

It has been assumed that the mechanism and efficiency of P digestion in animals will not experience interference by CP deficiency in a short period of time. However, in the current study, the results indicated that there was a limiting effect of CP deficiency on ileal P digestion in growing pigs, which indicated that CP deficiency decreased ileal P digestion. The present data also showed that the maximum P digestion was reached in the mid-high ATTDP diet in high CP group. Thus, there was a quadratic increase of digested P in the high CP diets with increasing dietary P concentrations. This quadratic pattern is reasonable because the content of P in the high ATTDP diet exceeded the requirement in NRC (1998). Theoretically, the amount of digested nutrient would increase with the dietary intake of that nutrient, until the capacity for absorption is reached. Therefore, a quadratic pattern of absorption can be observed. However, there was only a linear response in the low CP group, because the digested P did not reach the plateau until the high ATTDP diet. In other words, the maximum absorption of P was attained at a greater dietary P concentration in the low CP group. This result also suggested that low CP intake reduced the efficiency of P absorption. Additionally, the ATTDP concentration in the NFD was equivalent to 100% of requirement, which was the mid-high ATTDP concentration in this study. However, the digested P in NFD was 1.17 g/kg DMI, which is comparable to the result from the mid-low ATTDP diet in the low CP group (1.16 g/kg DMI). This phenomenon also suggested the efficiency of P digestion was limited by the lower dietary CP concentration.

The absorption of P in small intestine is affected by a series of factors, such as P concentration, Ca:P ratio, vitamin D, and the expression of sodium-phosphate co-transporter IIb (NaPi-IIb) (Saddoris et al., 2010; Adedokun et al., 2012; Liu et al., 2013). A plausible explanation for the decreased ileal P digestion in the low dietary CP group might be the limited AA intake impaired the active transportation system of P in the small intestine. The expression of NaPi-IIb, the main transporter in the P active transport system, will be upregulated when P concentration in the lumen of the small intestine is low (Saddoris et al., 2010; Adedokun et al., 2012). The NaPiIIb transporter protein synthesis or gene expression might be impaired in situations of AA deficiency (Xue et al. 2016), which could lead to the result of lower P digestion. Additionally, some AA are necessary to maintain gut health, such as threonine, a key component for mucin production (Law et al., 2007; Wang et al., 2007). Thus, insufficient AA could result in poor integrity and morphology of the microvilli. This may also contribute to the decreased P digestion in low CP diets. Further studies are needed to investigate this supposition.

The determined true ileal P digestibility of mono-calcium phosphate using the regression method were approximately 54, and 76% in the low and high CP diets, respectively. The true total tract digestibility of mono-calcium phosphate was reported to be 67.5% (Zhai and Adeola, 2012), which is close to the result determined with the high CP diets in the current study. Although statistical difference was not observed in this study, the 22 percentage unit numerical difference is still considerable if those values were referenced in practical diet formulation. Liu and Adeola (2016) indicated that the inclusion of casein in the basal diet does not affect the estimates of P digestibility determined by the regression method. The result in the present study supports that conclusion. However, the numerical difference was more dramatic in the current study, which may be a result of the severe protein deficiency in the low protein diets in the current study. Thus the results in this study indicated that the CP concentration in diets should be provided close to the requirement.

Dietary P concentration did not affect the digestion of N in the low CP group, but did interact with N digestion in the high CP group in a quadratic pattern. Thus, it is possible that dietary P concentrations could affect the digestion of protein, but further studies are needed to investigate the relationship. The effect of dietary ATTDP concentration on ileal AA digestibility was not observed in the current study. Based on the results of this study, it is reasonable to point out that the dietary CP concentration may affect the digestion of P in the small intestine, while P exerts little influence on ileal AA digestibility determination.

In the current study, it is not surprising that the main effect of dietary CP concentration on AID determination was observed. The calculation of AID of AA does not take endogenous loss of AA into account, which may lead to the underestimation of digestibility (Stein et al. 2007). This underestimation is even greater when the experimental diet contains low CP content, due to the relatively larger portion of endogenous loss in the total ileal AA flow. Thus it is well accepted that the dietary requirement for growing pigs and digestibility of AA in feed ingredients should be expressed on a standardized ileal digestible basis (Stein et al. 2007). The advantage of using SID of AA is the additivity in complete diets for animals (Stein et al. 2005; Kong and Adeola, 2013; Xue et al., 2014). To derive SID, BEL is adjusted from the AID.

Theoretically, the estimation of SID of AA in feed ingredients should be independent from the CP concentration of the experimental diets (Stein et al. 2007), whereas the result in the current study indicated otherwise. There was an effect of dietary CP concentration on the determination of SID for the experimental diets in this experiment where the low CP group had lower SID compared with the high CP group. This phenomenon seems to be in conflict with the definition of BEL and SID. However, previous studies also indicated that the estimation of SID of AA can be affected by the level of dietary CP content (Zhai and Adeola, 2011). Eklund et al. (2008) indicated that the endogenous loss of AA can be increased along with the increasing concentration of dietary protein. Considering the large difference in dietary CP content between these two groups, the physiological status might also be impacted due to the severe protein deficiency in the low CP group. Therefore, the estimation of digestibility from diets with different CP content might not be comparable. In addition, the BEL estimation in this study was relatively greater in most of the individual AA and total AA compared with previous reports (Nyachoti et al., 1997; Fan and Sauer, 2002; Moter and Stein, 2004; Zhai and Adeola, 2011), especially for Proline. The large endogenous loss of Proline led to a negative estimation of AID for Proline. This large endogenous AA flow can also be a factor that affected the calculation of SID in current study. These results indicated that although the estimation of SID should be independent from the dietary CP concentration, the experimental diet should be formulated at a CP content close to a normal practical diet for a comparable result.

In conclusion, our research emphasizes an important linkage between N and P digestion, the results showed CP deficiency limits ileal P digestion in growing pigs.

These two important aspects should be considered together in related studies of swine nutrition. Further studies into the response of P and AA transporters in small intestine to various CP and P concentrations are still needed to investigate the relationship between AA and P nutrition in pigs and its mechanism.

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Table 4-1. Ingredient and nutrient composition of diets

CP level[1], %	6.9				13.4				NFD[3]
Apparent total tract digestible P level[2],%	0.09	0.16	0.24	0.32	0.09	0.16	0.24	0.32	
Corn	210.0	210.0	210.0	210.0	440.0	440.0	440.0	440.0	0.0
SBM	100.0	100.0	100.0	100.0	200.0	200.0	200.0	200.0	0.0
Cornstarch	581.1	574.3	567.0	559.8	308.3	301.4	294.2	287.1	671.4
Dextrose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	200.0
Soy oil	30.0	30.0	30.0	30.0	15.0	15.0	15.0	15.0	30.0
Chromic oxide marker	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Monocalcium phosphate	2.2	5.6	9.3	12.9	0.0	3.5	7.1	10.7	11.4
Limestone	4.2	7.6	11.2	14.8	4.2	7.6	11.2	14.7	11.2
Solka-floc	40.0	40.0	40.0	40.0	0.0	0.0	0.0	0.0	40.0
Sodium Chloride	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Vitamin premix[5]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Mineral premix[6]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Selenium premix[7]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Potassium carbonate	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	4.0
Magnesium oxide	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.0
Calculated Nutrients & Energy									
Protein, g/kg	68.9	68.9	68.9	68.9	137.9	137.9	137.9	137.9	0.0
ME, kcal/kg	3833	3806	3776	3748	3884	3857	3828	3800	3659
Ca, g/kg	2.30	4.13	6.09	8.04	2.30	4.15	6.09	8.00	6.08
Total P	1.84	2.56	3.33	4.09	2.76	3.50	4.25	5.01	2.45
ATTD P[4], g/kg	0.91	1.63	2.40	3.16	0.90	1.64	2.39	3.15	2.39
Ca:P	2.52	2.54	2.54	2.54	2.55	2.54	2.55	2.54	2.54
NDF	77.3	77.3	77.3	77.3	76.9	76.9	76.9	76.9	38.8
ADF	15.3	15.3	15.3	15.3	30.7	30.7	30.7	30.7	0.0
Total amino acids, g/kg									
Arginine	4.3	4.3	4.3	4.3	8.6	8.6	8.6	8.6	0.0
Histidine	1.9	1.9	1.9	1.9	3.7	3.7	3.7	3.7	0.0
Isoleucine	2.8	2.8	2.8	2.8	5.7	5.7	5.7	5.7	0.0
Leucine	6.0	6.0	6.0	6.0	12.0	12.0	12.0	12.0	0.0
Lysine	3.7	3.7	3.7	3.7	7.4	7.4	7.4	7.4	0.0
Methionine	1.1	1.1	1.1	1.1	2.1	2.1	2.1	2.1	0.0
Phenylalanine	3.3	3.3	3.3	3.3	6.7	6.7	6.7	6.7	0.0
Threonine	1.9	1.9	1.9	1.9	3.8	3.8	3.8	3.8	0.0
Tryptophan	0.9	0.9	0.9	0.9	1.7	1.7	1.7	1.7	0.0
Valine	3.2	3.2	3.2	3.2	6.4	6.4	6.4	6.4	0.0

[1]Refers to low and high CP, respectively.

[2]Refers to low, med-low, med-high, and high ATTDP, respectively.

[3]Nitrogen free diet.

[4]Apparent total tract digestible P.

[5]Vitamin premix supplied per kg of diet: vitamin A, 2,423 IU; vitamin D_3, 242 IU; vitamin E, 17.6 IU; vitamin K activity, 2.4 mg; menadione, 804 μg; vitamin B_{12}, 14.1 μg; riboflavin, 2.8 mg; d-pantothenic acid, 9 mg; and niacin, 13 mg.

[6]Mineral premix supplied per kg of diet: Cu (as copper sulfate), 9 mg; I (as calcium iodate), 0.34 mg; Fe (as ferrous sulfate), 97 mg; Mn (as manganese oxide), 12 mg; and Zn (as zinc oxide), 97 mg.

[7]Supplied 300 μg of Se per kg of diet.

Table 4-2. Response of ileal digestion of P and nitrogen in growing pigs[1]

CP level[2], %	6.9				13.4					*P*-Value			Contrasts			
Apparent total tract digestible P level[3],%	0.09	0.16	0.24	0.32	0.09	0.16	0.24	0.32	SEM	CP level	P level	CP level × P level	Low CP linear P	Low CP Quad P	Hi CP linear P	Hi CP Quad P
Apparent ileal digestibility, %																
Dry matter	80.76	82.45	80.50	80.06	78.48	79.98	80.31	79.66	1.44	0.169	0.663	0.747	0.468	0.409	0.533	0.445
P	36.48	42.68	44.86	47.08	24.63	40.91	44.58	37.15	3.27	0.013	0.001	0.195	0.021	0.527	0.009	0.001
N	59.43	64.81	60.74	61.88	76.83	81.23	81.11	78.18	2.52	<0.001	0.240	0.769	0.728	0.341	0.709	0.136
Standardized ileal digestibility, %																
N	83.46	89.63	84.57	85.51	87.92	91.66	91.94	88.99	2.52	<0.001	0.240	0.769	0.728	0.341	0.709	0.136
Ileal digested nutrients, g/kg•DMI																
P	0.71	1.16	1.64	2.03	0.70	1.54	2.03	1.99	0.12	0.046	<0.001	0.141	<0.001	0.812	<0.001	0.002
Apparent ileal digested N	7.46	7.82	7.71	7.93	20.99	23.49	22.37	21.90	0.41	<0.001	0.010	0.045	0.402	0.850	0.354	0.001
Standardized ileal digested N	10.47	10.83	10.72	10.94	24.00	26.51	25.38	24.92	0.41	<0.001	0.010	0.045	0.402	0.850	0.354	0.001

[1]N= 6 replicates.

[2]Refers to low and high CP, respectively.

[3]Refers to low, med-low, med-high, and high ATTDP, respectively.

Table 4-3. Apparent ileal digestibility (AID) of nitrogen and amino acids in diets[1], %

CP level[2], %	6.9				13.4					*P* Values			Contrasts			
Apparent total tract digestible P level[3],%	0.09	0.16	0.24	0.32	0.09	0.16	0.24	0.32	SEM	CP level	P level	CP level × P level	Low CP linear P	Low CP quad P	Hi CP linear P	Hi CP quad P
Nitrogen	57.45	66.71	60.00	61.74	77.25	81.46	80.37	79.03	2.81	<.0001	0.120	0.712	0.598	0.157	0.737	0.329
Indispensable amino acids																
Arginine	74.90	79.46	74.36	76.39	88.28	89.81	89.62	88.83	1.77	<.0001	0.304	0.538	0.934	0.446	0.855	0.514
Histidine	78.97	81.08	77.86	77.33	85.82	88.18	87.49	87.21	1.48	<.0001	0.326	0.590	0.191	0.343	0.601	0.377
Isoleucine	77.46	78.92	74.90	76.49	83.07	85.86	85.40	83.34	1.46	<.0001	0.282	0.339	0.260	0.963	0.960	0.105
Leucine	79.64	81.91	77.80	78.68	84.44	87.16	86.64	85.60	1.64	<.0001	0.339	0.558	0.310	0.649	0.686	0.257
Lysine	80.97	81.86	79.17	79.47	85.46	89.11	88.18	87.45	1.44	<.0001	0.359	0.408	0.234	0.825	0.437	0.136
Methionine	77.62	79.84	78.87	79.43	84.89	88.41	87.02	86.75	1.72	<.0001	0.392	0.974	0.533	0.604	0.589	0.275
Phenylalanine	79.29	81.40	77.21	77.91	83.84	86.93	86.19	85.08	1.44	<.0001	0.180	0.401	0.171	0.601	0.649	0.154
Threonine	63.90	66.73	59.08	60.72	73.27	77.91	77.94	76.22	2.31	<.0001	0.243	0.145	0.080	0.784	0.396	0.176
Tryptophan	79.39	83.95	79.54	78.84	87.16	90.10	90.13	88.82	1.61	<.0001	0.097	0.456	0.367	0.085	0.490	0.193
Valine	73.68	74.59	71.58	71.70	80.26	83.15	82.66	80.55	1.84	<.0001	0.484	0.648	0.246	0.819	0.964	0.181
Dispensable amino acids																
Alanine	69.18	73.20	66.66	69.97	79.37	82.42	82.24	80.29	2.05	<.0001	0.266	0.358	0.625	0.852	0.778	0.230
Aspartic acid	77.09	78.45	74.18	74.50	81.82	84.87	84.59	82.41	1.40	<.0001	0.126	0.191	0.043	0.688	0.812	0.068
Cysteine	68.75	70.49	66.33	69.79	76.49	81.33	80.70	79.17	2.12	<.0001	0.424	0.408	0.909	0.664	0.438	0.139
Glutamic acid	83.14	84.62	81.57	82.09	87.71	89.40	89.37	88.13	1.20	<.0001	0.368	0.471	0.218	0.667	0.821	0.228
Glycine	29.89	48.51	33.76	36.85	70.58	73.29	73.10	73.39	5.25	<.0001	0.216	0.392	0.779	0.118	0.727	0.819
Proline	-76.49	-10.46	-54.47	-38.28	68.78	60.31	65.27	67.89	19.86	<.0001	0.484	0.285	0.396	0.183	0.980	0.781
Serine	72.33	75.17	69.34	69.56	81.01	83.83	83.33	81.53	1.72	<.0001	0.109	0.291	0.054	0.416	0.891	0.186
Tyrosine	71.83	74.65	66.40	70.55	83.98	86.95	86.20	84.44	1.68	<.0001	0.051	0.067	0.090	0.673	0.932	0.166
Total	66.23	72.77	65.97	67.74	82.21	84.24	84.21	83.09	2.18	<.0001	0.210	0.442	0.801	0.245	0.789	0.474

[1]N= 6 replicates.

[2]Refers to low and high CP, respectively.

[3]Refers to low, med-low, med-high, and high ATTDP, respectively.

Table 4-4. Endogenous amino acid losses at the terminal ileum of pigs fed nitrogen-free diet[1]

	Endogenous loss, mg/kg DMI		Ratio to endogenous loss of Lys[2]		AA composition, g/16g N	
Items	Mean	SE	Mean	SE	Mean	SE
Nitrogen	3,011	209	-	-	-	-
Indispensable amino acids						
Arginine	776	83	244	18	4.10	0.26
Histidine	171	22	53	1	0.91	0.08
Isoleucine	267	24	85	6	1.42	0.08
Leucine	468	55	146	7	2.47	0.18
Lysine	329	51	-	-	1.73	0.20
Methionine	63	7	20	1	0.33	0.02
Phenylalanine	281	34	87	4	1.49	0.12
Threonine	545	39	176	14	2.93	0.20
Tryptophan	95	7	30	2	0.51	0.03
Valine	398	33	128	11	2.15	0.20
Dispensable amino acids						
Alanine	626	56	198	12	3.32	0.16
Aspartic Acid	731	60	232	14	3.90	0.21
Cysteine	149	13	47	3	0.79	0.04
Glutamic Acid	886	79	280	16	4.72	0.27
Glycine	1,746	141	558	46	9.26	0.30
Proline	7,729	753	2,494	294	40.91	2.69
Serine	558	42	178	12	2.99	0.20
Tyrosine	229	26	72	4	1.21	0.09
Total amino acid	16,240	1373	-	-	-	-

[1]N= 6 replicates.

[2] Ratios are calculated by dividing the endogenous loss of each individual AA by the endogenous loss of lysine and multiplying 100.

Table 4-5. Standardized ileal digestibility (SID) of nitrogen and amino acid in diets[1], %

CP level[2], %	6.9				13.4					*P* Values			Contrasts			
Apparent total tract digestible P level[3],%	0.09	0.16	0.24	0.32	0.09	0.16	0.24	0.32	SEM	CP level	P level	CP level × P level	Low CP linear P	Low CP quad P	Hi CP linear P	Hi CP quad P
Nitrogen	81.48	91.53	83.83	85.37	88.34	91.89	91.20	89.84	2.81	0.016	0.111	0.555	0.734	0.110	0.764	0.387
Indispensable amino acids																
Arginine	91.30	96.69	92.40	93.66	96.28	97.29	97.13	96.43	1.77	0.009	0.314	0.549	0.707	0.216	0.972	0.632
Histidine	87.62	90.25	87.00	86.52	90.14	92.38	91.70	91.43	1.48	0.001	0.298	0.672	0.290	0.265	0.631	0.402
Isoleucine	85.55	87.60	83.55	84.89	87.33	89.82	89.38	87.39	1.46	0.003	0.252	0.448	0.326	0.795	0.970	0.133
Leucine	86.08	88.78	84.87	85.46	87.89	90.42	89.89	88.91	1.64	0.010	0.328	0.670	0.399	0.491	0.732	0.290
Lysine	88.45	89.97	87.24	87.59	89.34	92.74	91.83	91.15	1.44	0.004	0.323	0.587	0.376	0.665	0.485	0.164
Methionine	83.36	86.23	84.60	85.19	87.76	91.14	89.90	89.49	1.72	0.000	0.327	0.989	0.590	0.478	0.609	0.277
Phenylalanine	86.38	88.92	84.92	85.44	87.59	90.46	89.74	88.63	1.44	0.009	0.179	0.538	0.262	0.455	0.713	0.176
Threonine	81.62	85.84	78.86	79.87	82.80	86.90	86.80	85.26	2.31	0.017	0.230	0.332	0.209	0.457	0.485	0.229
Tryptophan	88.99	93.56	89.11	88.47	91.70	94.20	94.24	93.14	1.61	0.004	0.125	0.453	0.371	0.087	0.544	0.269
Valine	84.33	86.28	82.86	83.05	85.91	88.54	87.98	85.96	1.84	0.022	0.407	0.761	0.346	0.608	0.963	0.213
Dispensable amino acids																
Alanine	83.78	89.05	82.89	85.41	87.27	89.99	89.73	87.91	2.05	0.017	0.219	0.486	0.882	0.476	0.857	0.274
Aspartic acid	85.95	87.97	83.79	84.03	86.56	89.32	89.03	86.89	1.40	0.011	0.124	0.324	0.093	0.497	0.914	0.087
Cysteine	81.04	84.04	79.82	82.14	82.92	87.20	86.85	85.33	2.12	0.011	0.371	0.606	0.915	0.862	0.471	0.178
Glutamic acid	89.15	91.08	88.11	88.51	90.94	92.44	92.40	91.19	1.20	0.003	0.350	0.584	0.328	0.497	0.898	0.264
Glycine	81.10	103.34	90.30	91.79	98.40	99.67	99.14	99.92	5.25	0.037	0.161	0.245	0.386	0.039	0.865	0.964
Proline	90.86	165.55	130.00	138.07	155.46	143.74	148.00	150.80	19.86	0.179	0.432	0.181	0.204	0.078	0.913	0.716
Serine	86.82	90.57	85.66	85.47	88.80	91.38	90.80	89.12	1.72	0.017	0.138	0.574	0.215	0.224	0.962	0.223
Tyrosine	84.08	86.93	80.25	82.85	88.70	91.47	90.83	89.08	1.68	<.0001	0.117	0.206	0.144	0.938	0.948	0.187
Total	86.71	94.61	88.25	89.44	93.06	94.52	94.46	93.46	2.18	0.008	0.173	0.389	0.843	0.104	0.906	0.577

[1]N= 6 replicates.

[2]Refers to low and high CP, respectively.

[3]Refers to low, med-low, med-high, and high ATTDP, respectively.

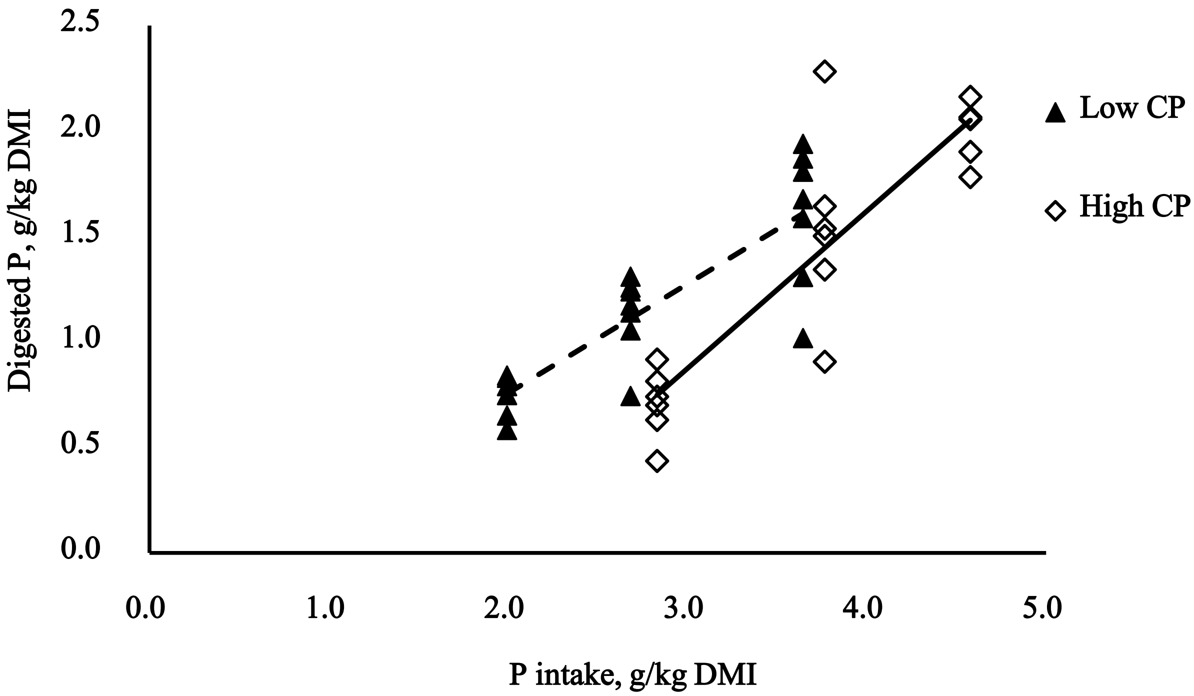

Figure 4-1. Ileal digested P (g/kg DMI) in low, med-low, and med-high ATTDP groups regressed against dietary P intake (g/kg DMI), for diets with low or high CP concentration. The statistical model was P_D = $Level_{CP}$ P_I $Level_{CP}$ *P_I, where P_D is ileal digested P (g/kg DMI); $Level_{CP}$ was coded as a dummy variable (0 for low CP diets; 1 for high CP diets) and included in the class statement; P_I is dietary P intake (g/kg DMI) and was not included in the class statement; and $Level_{CP}$ *P_I is the interaction of $Level_{CP}$ and P_I. The regression slope represented an estimation of true ileal P digestibility of mono-calcium phosphate. The slopes were not different from each other (P = 0.098). The overall P value for the regression model was < 0.001, R^2 = 0.788. The regression equation for the low CP group was Y = 0.544 (SE = 0.087, P < 0.001) X – 0.357 (SE = 0.271, P = 0.199). The regression equation for the high CP group was Y = 0.756 (SE = 0.088, P < 0.001) X – 1.412 (SE = 0.343, P < 0.001).

CHAPTER 5. PHOSPHORUS AND NITROGEN UTILIZATION RESPONSES OF BROILER CHICKENS TO DIETARY CRUDE PROTEIN AND PHOSPHORUS CONCENTRATIONS

5.1 Abstract

A study was conducted to investigate the effect of dietary CP concentrations on pre-cecal digestibility and total tract retention of phosphorus (P) in broiler chickens. A total of 384 14-d-old male broiler chickens were used in a randomized complete block design with 8 treatments and 6 replicates per treatment in a 7-d experimental period. There were 8 corn-soybean meal-based diets in a 2 × 4 factorial arrangement, which included 2 CP concentrations (10.7 or 21.5%) and 4 apparent total tract digestible P (ATTDP) concentrations (0.18, 0.32, 0.45, or 0.59%). Soybean meal and mono-calcium phosphate were used to adjust the CP and ATTDP concentrations, respectively. At the end of the experiment, BW was recorded and digesta samples from the distal two third of the ileum and mucosa samples from the middle of the jejunum was collected. Total RNA was also isolated from mucosa samples and used for real-time PCR to determine the gene expression of sodium-phosphate co-transporter IIb (NaPi-IIb). Results showed that a low dietary CP concentration limited the growth performance ($P < 0.01$), pre-cecal digestion and total tract retention of P ($P < 0.01$), and NaPi-IIb gene expression ($P < 0.05$), compared with a high dietary CP. Pre-cecal digestion and total tract retention of P (g/kg DM intake) linearly increased ($P < 0.01$) with increasing ATTDP concentrations in both low and high CP groups. In conclusion, this study suggests an interrelationship between N and P digestion such that CP deficiency decreased the growth performance of birds consequently reducing pre-cecal P digestion in broiler chickens. Total tract retention of CP and P are linked with each other and body tissue growth may be a driver of the deposition of these two nutrients. Supplementation of protein may be necessary in diets during P digestibility studies to ameliorate an effect of protein deficiency on P digestion and retention.

Key words: broiler chicken, crude protein, digestibility, phosphorus, retention

5.2 Introduction

For broilers, amino acids (AA) and phosphorus (P) are both essential nutrients playing roles in numerous biochemical reactions, such as protein synthesis and ATP production. The utilization of AA and P in feed is important in poultry nutrition, for which numerous studies have been conducted to determine the digestible content of AA and P in feed ingredients (Adebiyi and Olukosi, 2015; Chen et al., 2015; Dozier et al., 2015), and the factors affecting AA and P digestion (Iyayi et al., 2013; Adedokun and Applegate, 2014). However, in previous studies, AA and P digestibility have been considered separately. In studies determining P digestibility in feed ingredients, P concentration and Ca:P ratios in experimental diets are typically adjusted because they can impact P digestibility (Koshihara et al., 2004). However, dietary crude protein (CP) concentration is usually not considered a factor influencing P digestion, because it is assumed not to affect P digestibility. Likewise in AA digestibility studies, diets are typically maintained at similar CP concentrations as varying this may influence AA and N digestibility (Stein et al., 2007). However, the assumption that CP and P absorption are independent has not been well investigated, and the relationship between dietary protein concentration and P retention could be more complicated than previously assumed.

A strong correlation between whole body content of N and P was indicated in NRC (2012) in growing pigs. These data suggest that the amount of deposited N and P are related to each other. Although there is a lack of similar data in poultry, the relationship between N and P composition in broilers might be similar to that in growing pigs due to the similarity in nutrient requirements and the rapid growth rates of the two species. The relationship of protein and P could be linked in various aspects. For example, both lean tissue and bone development occur concurrently in growing animals, and both processes rely on an adequate supply of AA and P (Kimball et al., 2002; Escobar et al., 2006). In addition, AA and P may interact with each other at the concentration of absorption and utilization from the gut. The active transport of P in small intestine is dependent on the sodium-phosphate co-transporter IIb (NaPi-IIb), which is a protein synthesized in epithelial cells (Adedokun et al., 2012). A deficiency in AA may affect gut development and therefore affect the transport and utilization of P in the small intestine. Considering the related roles of protein and P in tissue growth and biochemical reactions, we hypothesized that an association exists between dietary AA concentration and P absorption and deposition in broilers. Therefore, there might be a relationship between dietary protein concentration and the digestion of P. Conversely, it is also possible that dietary P concentration could have an effect on N digestibility. Because this relationship has not been well defined in broiler chickens, this study was conducted with the objective of determining potential impact of dietary CP concentration on P digestion and retention in broiler chickens.

5.3 Materials and Methods

The experimental protocol used in this study was approved by the Purdue University Animal Care and Use Committee.

5.3.1 Birds and Diets

A total of three hundred and eighty-four male broiler chicks (Ross 708) were used in this study. The birds were maintained in cages in an environmentally controlled room and fed a standard starter diet (CP: 23%, non-phytate P: 0.45 %) that met all the recommended nutrient concentrations in manual of the breeder from d 0 till d 14 post hatching. On d 14, all birds were weighed individually, grouped by BW into 6 blocks, and randomly assigned to one of the 8 dietary groups in each block with 8 birds per cage. For the 2-wk starter phase before the experimental period, the average BW and total feed intake per bird were 374 and 457 g, respectively. Birds had free access to feed and water during the experimental period. Individual BW and feed consumption (per cage) were recorded at d 21 post-hatching. All birds received one of the 8 experimental diets arranged in a 2 x 4 factorial from d 14 to 21. Corn-SBM based diets were formulated in 2 CP concentrations that contained 10.7 or 21.5% CP of diet (low- or high-CP, equivalent to 50 and 100% of requirement). Within each CP concentration, 4 diets were formulated to contain apparent pre-cecal digestible phosphorus (ATTDP) concentrations of 0.18, 0.32, 0.45, and 0.59% of diet (equivalent to 40, 70, 100, and 130% of requirement, respectively). This arrangement of treatments also fit the statistical model for using the regression method to determine the digestibility of P in mono-calcium phosphate (MCP), because all the increase of P in the low- or high-CP diets was provided by MCP. The estimates of P digestibility generated by low- and high-CP diets can be compared to determine if CP concentration of the basal diet affects the regression-derived estimate of P digestibility in MCP. The composition of diets and concentrations of nutrients are shown in Table 5-1. Corn starch and mono-calcium phosphate were used to adjust the N and P concentration in diets. Limestone was included to maintain a similar Ca: non-phytate P ratio at 2:1. Chromic oxide was incorporated into the diets as indigestible marker at 5 g/kg on an as-fed basis. Solka-floc was added to the 4 low-CP diets to balance the concentration of dietary fiber.

5.3.2 Sample Collection

Excreta samples were collected twice daily from d 19 to 20 post hatching to calculate apparent N and P retention. On d 21, pre-cecal digesta was collected from the distal two thirds of the entire ileum, pooled per cage, and stored at -20 °C until analysis. Mucosa from the middle jejunum was collected from one bird per cage, stored in Trizol reagent (Invitrogen, Carlsbad, CA), frozen in liquid nitrogen, and subsequently stored at −80ºC. Total RNA was extracted from the mucosa samples according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). The concentration of RNA was determined using a NanoDrop ND-1000 UV-Vis spectrophotometer (Thermo Scientific, Wilmington, DE). Quantification of mRNA expression was determined on the MyiQ real-time PCR detection system (Bio-Rad, Hercules, CA), with SYBR green RT-PCR mix (SA Biosciences, Frederic, MD). Relative gene expression level was calculated by the comparative cycle threshold (CT) method (Livak and Schmittgen, 2001). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as the housekeeping gene. Primers used for the PCR are presented in Table 5-2.

5.3.3 Chemical Analyses

Pre-cecal samples were freeze-dried and excreta samples dried in a forced air oven at 56 °C to a constant weight, and ground to pass through a 0.5-mm screen before analysis. All samples were dried at 105 °C in a drying oven (Precision Scientific Co., Chicago, IL) for 24 h to determine DM content. Nitrogen content was determined by the combustion method (TruMac N analyzer; Leco Corp., St. Joseph, MI). Chromium content was determined by digesting the samples in concentrated nitric acid and 70% perchloric acid (method 935.13 A (a); AOAC International, 2000) and absorption was measured at

440 nm using a spectrophotometer (Spectronic 21D, Milton Roy Co., Rochester, NY). Phosphorus in samples was determined after wet digestion in nitric and perchloric acids (Fenton and Fenton, 1979). A blue color was developed by adding Fiske–Subbarow reducer solution and acid molybdate to digested samples, followed by measuring absorption using a spectrophotometer at 620 nm (Spectronic 21D; Milton Roy Co., Rochester, NY).

5.3.4 Calculation and Statistical Analysis

Apparent nutrient utilization (pre-cecal digestibility and overall retention) for N and P were calculated according to the following equation:

$$ANU = \left(1 - \left(\frac{C_I}{C_O} \times \frac{N_O}{N_I}\right)\right) \times 100$$

Where ANU is the apparent nutrient utilization (pre-cecal digestibility or overall retention of N or P); Cr_I is the chromium concentration in diets; Cr_O is the chromium concentration in the output (in pre-cecal digesta or excreta); N_I means CP or P concentration in diets; and N_O is CP or P output in pre-cecal digesta or excreta. The apparent pre-cecal digested or total tract retained N or P on g/kg DM intake (DMI) basis were calculated based on the N or P concentration in diet and corresponding coefficients of N or P.

The data were analyzed using the MIXED procedure of SAS (9.4) (SAS Institute, 2006). Fixed effects were N and P, block of BW was considered as a random effect, main and interaction effects of N and P were tested, as well as linear and quadratic response of SID to various P concentrations. The REG procedure of SAS (9.4) (SAS Institute, 2006) was used to analyze the relationship between apparent total tract N and P retention (g/bird). Apparent total tract P retention (g/bird) was regressed against apparent total tract N retention (g/bird). Polynomial regression was conducted if a higher power item was significant. An α concentration of 0.05 was used to assess significant differences.

Regression method was used to estimate the true digestibility of P in MCP. Analysis of covariance using proc GLM of SAS with solution option was used to estimate and compare the slopes for low and high diets. Pre-cecal digested P (g/kg DMI) was regressed against dietary P intake (g/kg DMI) for diets with low or high CP concentration. The statistical model was:

$$P_D = Level_{CP} \quad P_I \quad Level_{CP} * P_I,$$

where P_D is pre-cecal digested P (g/kg DMI); $Level_{CP}$ was coded as a dummy variable (0 or 1 for low- or high-CP diets, respectively) and included in the class variable; P_I is dietary P intake (g/kg DMI) and is not included in the class statement; $Level_{CP} *P_I$ is the interaction of $Level_{CP}$ and P_I. The slopes of the regression are estimates of true pre-cecal P digestibility. The *P*-value of the interaction term represents the difference of the two slopes.

5.4 Results

The results of final BW during the 7-d experimental period are presented in Table 5-3. Final BW of birds on the low CP diets containing 0.18, 0.32, 0.45, and 0.59% ATTDP were 479, 486, 496, and 515 g, respectively. Corresponding BW for birds fed the high CP diets were 717, 703, 709, and 715 g, respectively. The BW gain, feed intake, and gain:feed data are also presented in Table 5-3. Low CP diets limited the final BW, BW gain, feed intake, and gain:feed ratio in broilers from d 14 to 21 post hatching, compared with the high CP diets ($P < 0.01$). Generally, P concentration did not affect the growth performance in the birds except for gain:feed ratio ($P < 0.05$). However, in the low CP diets, the final BW, BW gain, feed intake, and gain:feed increased linearly with increasing P concentration ($P < 0.05$).

The diets in this study were formulated on ATTDP basis, which resulted in diets with different total P concentrations across the low and high CP groups (Table 5-4). For this reason, the discussion of P utilization will focus on digested P in g/kg DMI rather than digestibility coefficient.

Apparent pre-cecal digested P were 2.45, 3.17, 4.78, and 4.32 g/kg DMI for diets containing 0.18, 0.32, 0.45, and 0.59% ATTDP, respectively in the low CP diet, and 2.96, 3.79, 4.83, and 5.55 g/kg DMI for the high CP diet. Low dietary CP concentration decreased ($P = 0.01$) pre-cecal digested P (g/kg DMI). Apparent pre-cecal digested P in birds on high CP diets increased linearly with increasing concentration of P ($P < 0.01$), but tended to increase in a quadratic pattern ($P = 0.07$) for the low CP. Apparent total tract retention of P were 2.08, 2.76, 3.64, and 4.13 g/kg DMI for diets containing 0.18, 0.32, 0.45, and 0.59% ATTDP, respectively in the low CP diet, and 2.90, 3.54, 4.14, and 4.24 g/kg DMI for the high CP diet, respectively. Low dietary CP decreased apparent total tract P retention ($P < 0.01$). Increasing dietary P concentration linearly increased apparent total tract P retention with both low and high CP ($P < 0.01$), whereas there was a trend of quadratic increase with high CP ($P = 0.06$). In addition, jejunal NaPi-IIb gene expression was down regulated ($P < 0.05$) in birds fed the low CP diets.

Regressions for the low ($Y = 0.343$ (SE = 0.064) $X + 1.739$ (SE = 0.502) and high $Y = 0.488$ (SE = 0.074) $X + 0.988$ (SE = 0.612) CP groups are presented in Figure

5-1. While the 2 slopes are different from zero at $P < 0.001$, they are not different from each other.

Apparent pre-cecal digested N in the low CP diet containing 0.18, 0.32, 0.45, and 0.59% ATTDP were 19.15, 19.86, 19.98, and 19.05 g/kg DMI, respectively, but 34.16, 33.02, 33.77, and 33.44 g/kg DMI in the high CP diet (Table 5-5). Apparent total tract retention of N within the low CP group were 12.6, 14.55, 15.54, and 14.88 g/kg DMI; and 24.73, 22.64, 23.59, and 23.23 g/kg DMI in the high CP group. There was no main effect of P on the apparent pre-cecal digestibility of N, but the amount of pre-cecal digested N was affected by the dietary N intake ($P < 0.01$). There was a main effect of P concentration on apparent total tract N retention ($P < 0.05$) and an interaction between CP and P concentration on the amount of total tract P retention, where N retention increased ($P < 0.05$) with increasing P concentration in birds fed the low CP diets, but not in those that received the high CP diet.

The polynomial regression of apparent total tract P retention (g/bird) against apparent total tract N retention (g/bird) for the 8 treatments is shown in Figure 5-2. The regression of apparent total tract P retention (g/bird) on apparent total tract N retention (g/bird) for each of the four ATTDP concentrations is shown in Figure 5-3. Polynomial regression equations for the four ATTDP groups are presented in the captions for Figure 5-3.

5.5 Discussion

Although the experimental period was only 7 d long, an improvement in growth performance of birds fed the high compared with the low CP diet was observed in the current study. In addition, the ATTDP concentration in the low CP group linearly increased BW gain, feed intake, and feed efficiency. This observation agrees with previous studies using regression methods, where increasing concentrations of CP and P improved growth performance of broilers in short-term studies (Dilger et al., 2004; Dilger and Adeola, 2006; Iyayi et al., 2013; Liu et al., 2014). Body tissue deposition can be considered a result of both N and P deposition. In the NRC (2012) for swine, it was concluded that whole body composition of N and P are highly correlated. This relationship suggests that, for animals, N and P are deposited simultaneously.

In studies focusing on P digestibility, dietary N concentration would not usually be considered as a factor affecting P digestion because it is assumed that the efficiency of P digestion in animals is not affected by N deficiency in the short term. However, results from the current study indicated that N may have an effect of limiting pre-cecal P digestion in diets deficient in CP. This indicates that N deficiency may decrease pre-cecal P digestion. In the current study, apparent pre-cecal digested P (g/kg DMI) was limited by CP deficiency. The results in Table 5-4 also showed that the pre-cecal digested P in the low CP group did not increase from mid-high to high P diets, where a plateau of P absorption was observed. As a result, the digested P in the low CP groups tended to increase in a quadratic pattern, rather than just a linear increase with rising dietary P concentrations. On the contrary, pre-cecal digested P in the high CP diets increased linearly in response to the increasing ATTDP concentration. This phenomenon indicated that P absorption in the small intestine of broilers may be impaired by dietary protein deficiency. The P digestibility of MCP determined by using the regression method showed no statistical difference between the two slopes. Previous studies suggested that basal diet type does not affect regression-derived estimates of P digestibility (Liu et al.

2014; Shastak et al. 2014), which is in agreement with the current study. However, a noteworthy result in the current study is that the estimate of P digestibility in MCP was lower than expected. One possible reason for this might be setting of P concentrations in diets. The objective of the current study was not to determine the P digestibility using the regression method. Thus, the P concentrations were not formulated to the ideal range and intervals for the application of regression. Rodehutscord et al. (2012) summarized that the response of pre-cecal digestion of P to dietary P concentrations was in a linear pattern between 2 to 7 g/kg of diet. Yet in the current study, the P concentrations exceeded that range in the high end. It is possible that the digestion of P was limited by high dietary P.

The main site of P absorption in the small intestine is the jejunum. Phosphorus can be transported across the enterocyte by either passive diffusion or active transport via the NaPi-IIb transporter (Huber et al., 2006; Saddoris et al., 2010). Under normal physiological circumstances, the expression of the NaPi-IIb gene in the small intestine is expected to be upregulated when dietary P is low (Olukosi et al., 2011; Nie et al., 2013). The reason being that availability of P_i is the main regulator of NaPi-IIb gene expression (Huber et al. 2015). This response is considered an adaptive response of the animal to P deficiency. In the current study, the effect of ATTDP concentration on jejunum NaPi-IIb expression was not observed. This may be attributed to the relatively high variation in the data. Nevertheless, there was an effect of CP concentration on the gene expression of NaPi-IIb in the jejunum. Although NaPi-IIb is just one of the P transporters in the small intestine, the decreased expression in the low P group can be linked with the P digestion data, where we observed decreased P absorption in the low CP group. Because availability of P_i in the small intestine is a key regulator for the expression of NaPi-IIb

(Huber et al. 2015), phytate content of the high CP diets may be a confounding factor. It is possible that the increased phytate in the high CP diets, mainly from corn and SBM, could affect the availability of P_i in the lumen of the small intestine. Further studies are needed as data in the current study is insufficient to establish this possibility.

Deficiency of CP also limited apparent total tract P retention in the current study. Compared with pre-cecal P digestion, the response of retained P (g/kg DMI) to increasing ATTDP concentration was different. The total tract retained P in the low CP diets linearly increased in response to the 4 ATTDP concentrations. The retention of P in broiler chickens can be affected by several factors, especially Ca:P ratio (Dilger et al., 2004; Stein et al., 2011; Liu et al., 2013). For this reason, it is recommended to use pre-cecal digestible basis for the expression of P utilization in broilers (Iyayi et al., 2013; Liu et al., 2013). In the current study, a constant Ca:digestible P ratio was maintained across all diets. Thus, the differences in P retention is more likely to be attributable to the BW gain and N retention, rather than Ca:P ratio. The BW gain and total tract N retention in the low CP group increased linearly with the increasing ATTDP concentrations, which is consistent with the linear increase of P retention. But N retention and BW gain were not increased by the high P diet, similar to the results in P retention. The capacity for P retention in birds fed diet containing the high CP and high P may have already been maximized.

There is a lack of studies investigating the relationship between ATTDP concentration and N digestibility. The effect of ATTDP concentration on N digestibility was not statistically significant in the current study. Although there was an interaction of CP and P concentration on N digestibility and greater ATTDP concentration affected N digestibility in a quadratic pattern within the low CP groups, it is unclear how the dietary ATTDP concentration affected the digestion of N. However, ATTDP concentration affected N retention in the current study, especially in the low CP diets, where the apparent total tract retention of N increased linearly with increasing dietary P. The results of N and P retention were in agreement with the growth performance data. Higher ATTDP concentrations increased the BW gain linearly in the low CP diets. As a result, the deposition of N was increased as well. Thus, these results suggest that body tissue gain may be the driver of N and P retention in broiler chickens, and that the deposition of N and P may be interlinked through contributions to body tissue growth.

The regression results of this study suggested a polynomial relationship between N and P retention. Similar to the result in NRC (2012), the polynomial regression showed that the relationship between N and P retention is complicated. However, it was clearly demonstrated that within each ATTDP concentration, the retention of P was linearly correlated with the retention of N. In addition, slopes of the linear equations were all close to 0.1 in the current study. This phenomenon suggested that there could be a quantitative relationship between N and P retention, which may be approximately 10:1 in broiler chickens. Although there is no literature data that investigated this relationship, results from other previous studies may shed light on this point of view. A review of data from studies that determined the effect of enzymes on nutrient retention (Dilger et al., 2004; Esmaeilipour et al., 2011; Naves et al., 2015) revealed that the average ratio between N and P retention (g:g) in young broiler chickens was 10.39:1 (a total of 16 observations, SD = 2.15), which is in agreement with the current study. However, a similar study conducted in growing pigs reported by Adeola et al. (2015) showed a different result, where increased digestible P concentration only elevated total tract P retention but not retained N. Although the study was designed to determine the digestibility of P in growing pigs with fixed CP concentration and increasing digestible P concentrations, the N and P retention data were comparable to the current experiment. But it should be noted that in the pig study, the feed intake of the experimental animals was restricted because the total collection method was applied. Therefore, the retention of N and P may not be in an ideal condition to investigate the relationship between N and P retention. The relationship between whole body composition of N and P in growing pigs summarized by NRC (2012) was generated by using pigs under normal feeding circumstances. Thus, the variation between studies cannot be simply considered to be the difference between swine and poultry. This difference may suggest that to investigate the relationship between N and P retention, feed intake should be set to a concentration that is close to ad libitum. The number of studies measuring N and P retention simultaneously are limited, because the utilization of and requirement for P and protein, or AA are usually investigated separately. Therefore, further studies are necessary to determine the potential quantitative relationship between N and P deposition over a longer period of growth.

Nutrient-deficient semi-purified diets may have a negative effect on nutrient digestibility. In the current study, the low CP diets decreased the BW gain and feed intake of the experimental animals, as well as compromised the absorption and utilization of nutrients. To ameliorate the potential negative effects, supplementation of diets with casein have been suggested (Adedokun et al., 2014; Liu et al., 2014; González-Vega et al., 2015). The findings in the current study support the notion that severe protein deficiency can impair the absorption of P in broiler chickens. Therefore, it may be necessary to provide sufficient protein in diets during P digestibility studies. However, it has been shown that the addition of casein, in some cases, did not affect the result of P digestion (Liu et al., 2014). Therefore, further studies are necessary to investigate the effect of casein addition to a protein deficient diet on P absorption.

In conclusion, this study suggests an interrelationship between N and P digestion such that CP deficiency decreased the growth performance of birds consequently reducing the pre-cecal P digestion in broiler chickens. Total tract retention of CP and P are linked with each other and body tissue growth may be a driver of the deposition of these two nutrients. Supplementation of protein may be necessary in diets during P digestibility studies to ameliorate an effect of protein deficiency on P digestion and retention.

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Table 5-1. Dietary composition and calculated nutrient content

CP level[1], %	10.7				21.5			
ATTDP level[2], %	0.18	0.32	0.45	0.59	0.18	0.32	0.45	0.59
Corn	263.7	263.7	263.7	263.7	527.3	527.3	527.3	527.3
SBM	175.0	175.0	175.0	175.0	350.0	350.0	350.0	350.0
Cornstarch	477.9	467.2	456.5	445.8	32.4	21.5	10.8	0.0
Soy oil	0.0	0.0	0.0	0.0	50.0	50.0	50.0	50.0
Mono-calcium phosphate[3]	5.8	12.2	18.6	25.0	2.9	9.4	15.8	22.2
Calcium carbonate[4]	5.7	10.0	14.3	18.6	5.4	9.8	14.1	18.5
Sodium chloride	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin-mineral premix[5]	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Cr_2O_3 premix[6]	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Solka-floc	40.0	40.0	40.0	40.0	0.0	0.0	0.0	0.0
Total	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Analyzed Nutrients,								
Crude protein, g/kg	119.1	133.7	143.0	133.5	225.8	227.7	229.3	220.4
Ca, g/kg	3.9	6.8	9.9	12.5	3.7	6.8	9.5	12.7
Total P, g/kg	3.2	4.6	6.8	8.8	4.1	5.9	7.3	9.1
Calculated Nutrients & Energy								
ME, kcal/kg	3,357	3,313	3,270	3,226	3,274	3,230	3,186	3,142
ATTD P, g/kg	1.8	3.2	4.5	5.9	1.8	3.2	4.5	5.9
Ca:ATTDP	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Total amino acids, g/kg								
Arginine	7.1	7.1	7.1	7.1	14.2	14.2	14.2	14.2
Histidine	2.8	2.8	2.8	2.8	5.7	5.7	5.7	5.7
Isoleucine	4.5	4.5	4.5	4.5	8.9	8.9	8.9	8.9
Leucine	9.2	9.2	9.2	9.2	18.4	18.4	18.4	18.4
Lysine	5.9	5.9	5.9	5.9	11.7	11.7	11.7	11.7
Methionine	1.6	1.6	1.6	1.6	3.3	3.3	3.3	3.3
Phenylalanine	5.1	5.1	5.1	5.1	10.2	10.2	10.2	10.2
Threonine	4.0	4.0	4.0	4.0	8.1	8.1	8.1	8.1
Tryptophan	1.5	1.5	1.5	1.5	2.9	2.9	2.9	2.9
Valine	4.9	4.9	4.9	4.9	9.9	9.9	9.9	9.9

[1]The two CP levels were equivalent to 50 and 100% of requirement, respectively.

[2]Apparent total tract digestible P. The four levels of ATTDP were equivalent to 40, 70, 100, and 130% of requirement, respectively.

[3]16 % Ca, 21 % P.

[4]38 % Ca.

[5]Supplied the following per kilogram of diet: vitamin A, 5,484 IU; vitamin D_3, 2,643 ICU; vitamin E, 11 IU; menadione sodium bisulfite, 4.38 mg; riboflavin, 5.49 mg; d-pantothenic acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B_{12}, 13.2 μg; biotin, 55.2 μg; thiamine mononitrate, 2.2 mg; folic acid, 990 μg; pyridoxine hydrochloride, 3.3 mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 300 μg.

[6]Prepared as 1 g of chromic oxide added to 4 g of corn.

Table 5-2. Primers used for real-time PCR[1]

Gene	Primer (5′-3′)
GAPDH (forward)	AGGGATGACTTTCCCTACAGCCTT
GAPDH (reverse)	ATGACCACTGTCCATGCCATCA
NaPi-IIb (forward)	CTGCAGGACACTGGAGTCAA
NaPi-IIb (reverse)	CCGCAACAGGATTAGAGAGC

[1]GAPDH = glyceraldehyde-3-phosphate dehydrogenase, NaPi-IIb = sodium-dependent phosphate transporter type II-b.

Table 5-3. Growth performance response to CP and P concentrations of broiler chickens

Item	Final BW, g/bird	BW gain, g/bird	Feed intake, g/bird	Gain:Feed, g:g
Low CP diets, 10.7 g/kg CP				
0.18% ATTDP[1]	478.8	91.9	351.5	262.1
0.32% ATTDP	486.2	104.4	373.8	278.0
0.45% ATTDP	495.7	111.2	373.8	296.4
0.59% ATTDP	514.9	130.2	396.3	327.9
High CP diets, 21.5 g/kg CP				
0.18% ATTDP	716.7	332.6	511.7	651.0
0.32% ATTDP	702.9	318.0	500.8	635.8
0.45% ATTDP	708.6	324.4	504.2	642.8
0.59% ATTDP	715.0	330.6	504.0	655.3
SEM	10.7	10.7	14.5	13.2
P-value for main effects and interaction				
CP level	<0.001	<0.001	<0.001	<0.001
P level	0.259	0.273	0.639	0.039
CP level × P level	0.372	0.305	0.357	0.146
P-value for contrasts of P level				
Low CP linear P	0.019	0.016	0.046	0.001
Low CP quadratic P	0.588	0.763	0.994	0.557
High CP linear P	0.989	0.994	0.763	0.740
High CP quadratic P	0.352	0.340	0.717	0.301

[1]Calculated apparent total tract digestible P, equivalent to 40, 70, 100, and 130% of requirement, respectively.

Table 5-4. Apparent pre-cecal and total tract utilization of P and jejunum NaPi-IIb mRNA expression in broilers fed with diets containing different CP and P concentrations

Item	Pre-cecal digestion		Total tract retention		Jejunum NaPi-IIb,
	Coefficient	Digested, g/kg DMI	Coefficient	Retained, g/kg DMI	
Low CP diets, 10.7 g/kg CP					
0.18% ATTDP[3]	0.69	2.45	0.58	2.08	1.42
0.32% ATTDP	0.62	3.17	0.54	2.76	0.94
0.45% ATTDP	0.64	4.78	0.49	3.64	0.88
0.59% ATTDP	0.44	4.32	0.43	4.13	0.93
High CP diets, 21.5 g/kg CP					
0.18% ATTDP	0.64	2.96	0.63	2.90	1.86
0.32% ATTDP	0.59	3.79	0.55	3.54	1.66
0.45% ATTDP	0.59	4.83	0.51	4.14	1.73
0.59% ATTDP	0.55	5.55	0.42	4.24	1.13
SEM	0.03	0.31	0.02	0.14	0.31
P-value for main effects and interaction					
CP level	0.326	0.010	0.163	<0.001	0.017
P level	<0.001	<0.001	<0.001	<0.001	0.303
CP level × P level	0.055	0.322	0.563	0.062	0.725
P-value for contrasts of P level					
Low CP linear P	<0.001	<0.001	<0.001	<0.001	0.287
Low CP quadratic P	0.054	0.065	0.659	0.518	0.400
High CP linear P	<0.001	<0.001	<0.001	<0.001	0.138
High CP quadratic P	0.060	0.860	0.958	0.062	0.521

[1]Mucosa samples were taken from one bird per cage. The housekeeping gene used was GAPDH. All values are in arbitrary units. GAPDH = glyceraldehyde-3-phosphate dehydrogenase, NaPi-IIb = sodium-dependent phosphate transporter type II-b.

[2]Calculated apparent total tract digestible P, equivalent to 40, 70, 100, and 130% of requirement, respectively.

Table 5-5. Apparent pre-cecal and total tract utilization of N in broilers fed with diets containing different CP and P concentrations

Item	Pre-cecal digestion		Total tract retention	
	Coefficient	Digested, g/kg DMI	Coefficient	Retained, g/kg DMI
Low CP diets, 10.7 g/kg CP				
0.18% ATTDP[1]	0.82	19.15	0.54	12.60
0.32% ATTDP	0.85	19.86	0.62	14.55
0.45% ATTDP	0.85	19.98	0.66	15.54
0.59% ATTDP	0.81	19.05	0.64	14.88
High CP diets, 21.5 g/kg CP				
0.18% ATTDP	0.85	34.16	0.61	24.73
0.32% ATTDP	0.82	33.02	0.56	22.64
0.45% ATTDP	0.84	33.77	0.59	23.59
0.59% ATTDP	0.83	33.44	0.58	23.23
SEM	0.01	0.37	0.02	0.48
P-value for main effects and interaction				
CP level	0.911	<0.001	0.007	<0.001
P level	0.213	0.387	0.018	0.175
CP level × P level	0.033	0.090	<0.001	<0.001
P-value for contrasts of P level				
Low CP linear P	0.851	0.909	<0.001	0.001
Low CP quadratic P	0.003	0.036	0.001	0.010
High CP linear P	0.465	0.399	0.196	0.104
High CP quadratic P	0.340	0.271	0.162	0.079

[1]Calculated apparent total tract digestible P, equivalent to 40, 70, 100, and 130% of requirement, respectively.

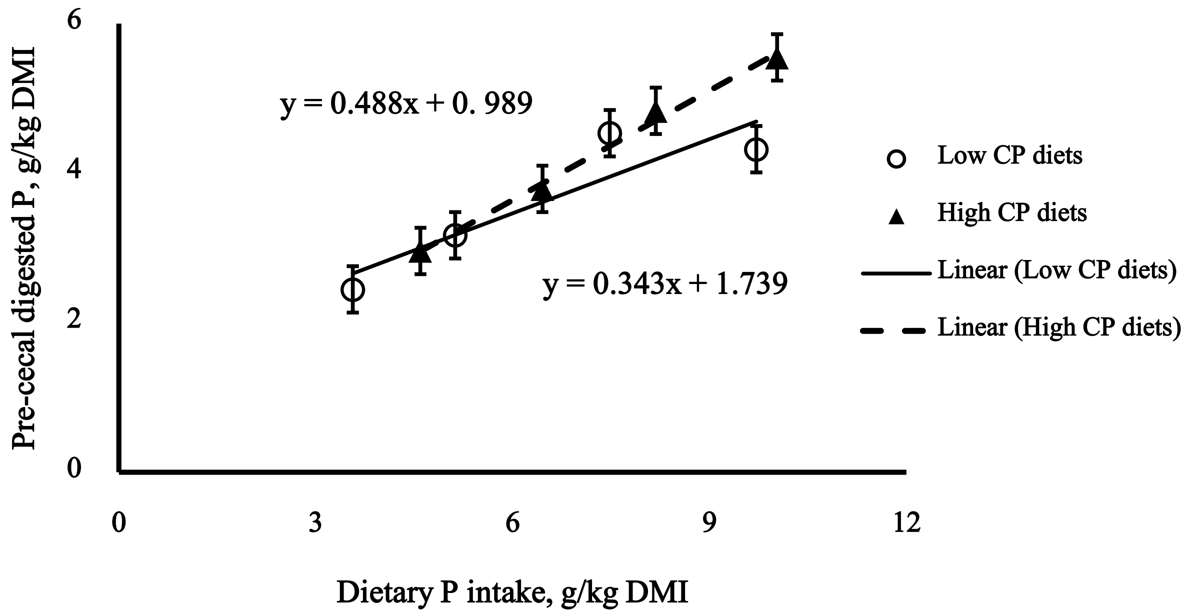

Figure 5-1. Pre-cecal digested P (g/kg DMI) regressed against dietary P intake (g/kg DMI), for diets with low (10.7%) or high (21.5%) CP concentration. The statistical model was $P_D = Level_{CP}\ P_I\ Level_{CP}\ {*}P_I$, where P_D is pre-cecal digested P (g/kg DMI); $Level_{CP}$ was coded as a dummy variable (0 for low CP diets; 1 for high CP diets) and included in the class statement; P_I is dietary P intake (g/kg DMI) and was not included in the class statement; and $Level_{CP}\ {*}P_I$ is the interaction of $Level_{CP}$ and P_I. The regression slope represented an estimation of true pre-cecal P digestibility. The slopes were not different from each other (P = 0.15). The overall P value for the regression model was < 0.001, R^2 = 0.693. The regression equation for the low CP group was Y = 0.343 (SE = 0.064, P < 0.001) X + 1.739 (SE = 0.502, P < 0.01). The regression equation for the high CP group was Y = 0.488 (SE = 0.074, P < 0.001) X + 0.988 (SE = 0.612, P > 0.05).

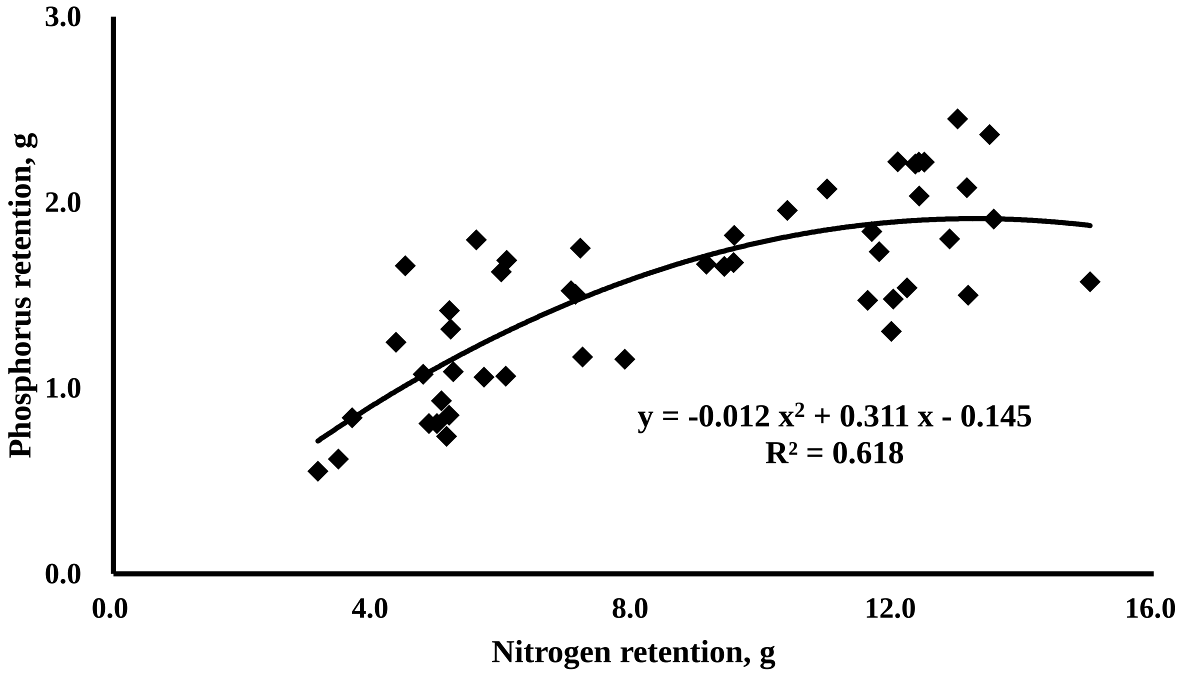

Figure 5-2. Relationship between apparent total tract N and P retention (g/bird). Each data point stands for a replicate cage. Apparent total tract P retention (g/bird) was regressed against apparent total tract N retention (g/bird). The polynomial regression equation was Y = -0.012 (SE = 0.005, $P < 0.05$) X^2 + 0.752 (SE = 0.094, $P < 0.01$) X – 1.279 (SE = 0.365, $P = 0.69$), $R^2 = 0.618$.

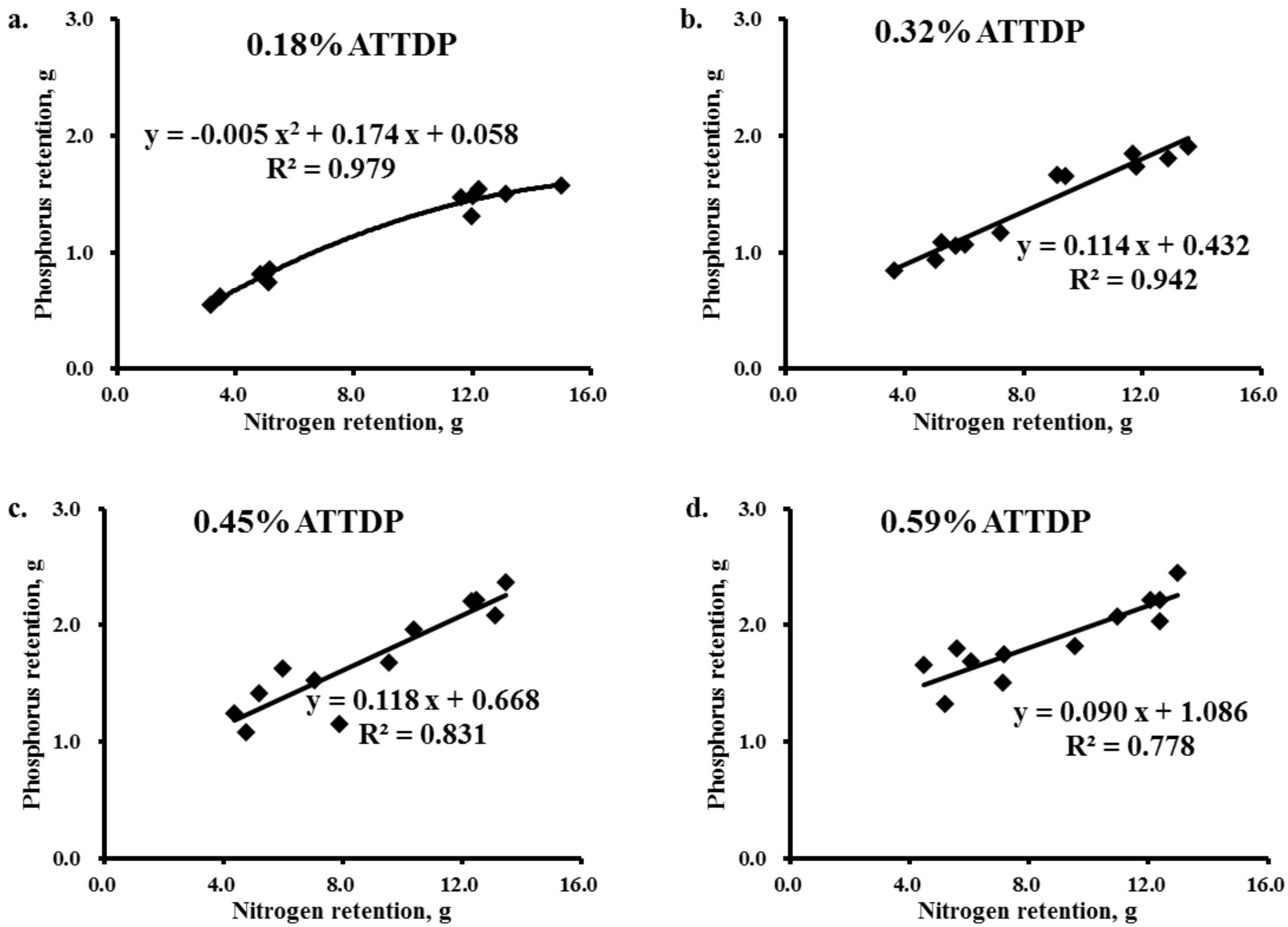

Figure 5-3. Relationship between apparent total tract N and P retention (g/bird) in each of the four apparent total tract digestible P (ATTDP) concentrations. Each data point stands for a replicate cage. Apparent total tract P

retention (g/bird) was regressed against apparent total tract N retention (g/bird). Polynomial regression was conducted if higher power item was significant. **Panel a.** The polynomial regression equation for the 0.18% ATTDP group was Y = -0.005 (SE = 0.002, $P < 0.05$) X^2 + 0.174 (SE = 0.035, $P < 0.01$) X + 0.058 (SE = 0.122, $P = 0.65$), $R^2 = 0.979$. **Panel b.** The regression equation for the 0.32% ATTDP group was Y = 0.114 (SE = 0.009, $P < 0.01$) X + 0.432 (SE = 0.081, $P < 0.01$), $R^2 = 0.942$. **Panel c.** The regression equation for the 0.45% ATTDP group was Y = 0.118 (SE = 0.017, $P < 0.01$) X + 0.668 (SE = 0.159, $P < 0.01$), $R^2 = 0.831$. **Panel d.** The regression equation for the 0.59% ATTDP groups was Y = 0.090 (SE = 0.015, $P < 0.01$) X + 1.086 (SE = 0.142, $P < 0.01$), $R^2 = 0.778$.

CHAPTER 6. INFLUENCE OF DIETARY CRUDE PROTEIN AND PHOSPHORUS CONCENTRATIONS ON THE UTILIZATION OF CRUDE PROTEIN AND PHOSPHORUS IN GROWING PIGS

6.1 Abstract

A study was conducted to determine the response of total tract utilization of CP and P to different CP and P concentrations in growing pigs. A total of 72 growing pigs (initial BW 20.9 ± 0.8 kg) were used in a randomized complete block design, with 9 treatments and four 10-d experimental periods giving 8 replicates per treatment. The pigs were blocked by BW and allotted to 9 treatments with a 3 × 3 factorial arrangement consisted of 3 CP concentrations (5.5, 9.7, or 13.9%) and 3 apparent total tract digestible P (ATTDP) concentrations (0.11, 0.19, or 0.27%). The CP concentration and ATTDP concentrations were adjusted using SBM and mono-calcium phosphate (MCP), respectively. Limestone was included to maintain a Ca: ATTDP ratio of 2.2 across diets. There was a 5-d adjustment period followed by a 5-d total collection period. Chromic oxide and ferric oxide were used as markers to time the initiation and termination of fecal collection, respectively. The daily feed intake was adjusted to 4% of the average BW of each block, in two equal daily feeding regimen at 0730 and 1730 h. Data was analyzed using SAS (9.4) and contrasts were used to test the linear and quadratic effects of increasing concentrations of P within each CP concentration, or vice versa. Digested P (g/d) was regressed against P intake (g/d) for each CP concentration to determine the true total tract digestibility (TTTD) of P in MCP. Digested P (g/d) increased linearly along with the increasing concentration of CP (*P* < 0.05). The determined TTTD of P in MCP for 5.5, 9.7, and 13.9% CP diets were 80.5, 82.6, and 87.9%, respectively. There were no statistical differences among the three TTTD estimates. In the nitrogen utilization results, increasing dietary P concentration decreased the urine nitrogen output (*P* < 0.05). In conclusion, the results indicated that dietary CP deficiency may limit total tract P digestion and retention. Yet the quantitative relationship between total tract N and P retention remains unclear.

Key words: crude protein, phosphorus, pigs, total tract digestibility, retention

6.2 Introduction

The protein and phosphorus (P) requirements are of great importance for the diet formulation of growing pigs. For studies evaluating the digestion and retention of N or P, these two aspects were usually considered separately. It was assumed that the dietary CP and P concentrations can not affect the digestion of each other in animals. However, studies have indicated that the concentration of CP and P in the diet might have effects on the digestion or retention of each other. In the NRC (2012), the P requirement for growing pigs was estimated based on the correlation of a relationship between whole body composition of N and P. It was reported that the dietary CP deficiency in experimental diets may limit P digestion in growing pigs and broiler chicken (Xue et al., 2015, 2016). In addition, gene expression of the sodium-phosphate co-transporter IIb (NaPi-IIb) can be limited by protein deficiency in the diet (Xue et al., 2016). In another study, Mutucumarana et al. (2015) reported a decreased estimation of true P digestibility determined by the regression method when egg albumen was included in the basal diet. Although the results in previous studies were not consistent, the effects of dietary CP concentration on P digestion were observed in previous studies.

On the other hand, dietary P exerts very little influence on the determination of digestibility of CP and AA in animals (Xue et al., 2015). Although the effect of dietary P concentration on AA digestibility was not observed, it is possible that the utilization of the digested CP and AA may be impacted by the P nutrition of the animal. In fact, the amino acids (AA) and P are widely related to each other in plenty of biochemical reactions and biosynthesis. The phosphorylation reaction is a common regulation reaction for different signaling pathways including protein syntheses (Sarbassov et al., 2005). Yet the effect of dietary P concentration on the utilization of CP and AA in growing pigs is unknown.

Considering the wide linkage between protein and P in animals, it is reasonable to believe there could be a quantitative correlation between the depositions of these two nutrients in the animal body. The investigation of this relationship can be helpful for the estimation of AA and P requirements of animals. A previous study from our lab demonstrated there is a quantitative correlation between the apparent total tract retention (ATTR) of N and P in broiler chickens (Xue et al., 2016). There is a lack of study focusing on the relationship between the ATTR of N and P in growing pigs.

The regression method can be applied to determine the true digestibility of P in feed ingredients. The formulation of the basal diet can impact the determination of digestibility of P in some scenarios (Liu et al., 2014a; Mutucumarana et al., 2015). But, there is a limited amount of research focused on the effect of dietary CP concentration in the basal diet to the determination of apparent (ATTD) and true (TTTD) total tract digestibility of P in growing pigs. Therefore, the objective of the current study was to determine the influence of dietary CP concentration on the estimation of ATTD and TTTD of P using the regression method; and to determine the quantitative relationship between the ATTR of N and P in growing pigs.

6.3 Materials and Methods

The experimental protocol used in this study was approved by the Purdue University Animal Care and Use Committee (PACUC).

6.3.1 Animals and sample collection

A total of 72 growing pigs (initial BW 20.9 ± 0.8 kg) were used in this study and allotted to a randomized completely blocked design, with 9 dietary treatments and four 10-d experimental periods. In each experimental period, there was a 5-d adjustment period followed by a 5-d total collection period. The pigs were housed individually in 18 stainless steel metabolism crates equipped with a feeder and a trough and nipple drinker. At the beginning of each period, the pigs were blocked by BW and assigned to 9 treatments with a 3 × 3 factorial arrangement. There were 8 replicates within each treatment after the four periods. The daily feed intake allowance was adjusted to 4% of the average BW of each block, in two equal meals fed twice daily at 0730 and 1730 h. Marker to marker approach, as described by Zhai and Adeola (2012), was applied by using chromic oxide and ferric oxide as the initiation and termination maker of fecal collection, respectively. Fecal samples were frozen at -20°C immediately after collection. Urine samples were collected proportionally everyday starting at the morning of d-6 and ending at the morning of d-11 of each period. Formaldehyde (20%) was added to collection vessels in 10 ml aliquots daily to prevent microbial activity in the urine samples.

6.3.2 Dietary Treatments

The 9 dietary treatments with 3 × 3 factorial arrangement consisted of 3 CP concentrations (5.5, 9.7, or 13.9%) and 3 apparent total tract digestible P (ATTDP) concentrations (0.11, 0.19, or 0.27%). For the convenience of expression, the three concentrations of CP and ATTDP were noted as low, medium, and high in this manuscript, respectively. The dietary formulation and calculated nutrient composition is presented in Table 6-1. The CP concentration was manipulated using corn and SBM, while mono-calcium phosphate (MCP) was used to adjust the ATTDP concentration. Limestone was included to maintain a Ca: ATTDP ratio of 2.2 across diets. Solka-floc was added in low and medium concentration CP diets to balance the fiber content. Within each CP concentration, the change of ATTDP concentration was solely contributed by the inclusion rate of MCP. Thus, the regression method can be applied to estimate the TTTD of P in MCP within each CP concentration. In other word, this study can also be considered as an investigation of the effect of the basal diet CP concentration on the determination of TTTD of P by using the regression method.

6.3.3 Chemical Analyses

All the collected fecal samples were dried at 55°C in a forced-air oven to constant weight. Diet and dried fecal samples were ground using a mill grinder (Retsch ZM 100, Retsch GmbH and Co., K.G., Haan, Germany) to pass through a 1.0-mm screen before analysis. The DM of diet and fecal samples were determined by drying in a forced-aired oven (Precision Scientific Co., Chicago, IL) for 24 h at 105°C [Method 934.01, (AOAC, 2006)]. The N content in diets, urine, and fecal samples was analyzed by using Leco TruMac Nitrogen Combustion Analyzer [LECO Corp., St. Joseph, MI; Method 990.03, (AOAC, 2006)]. The concentration of chromium and P in diet, urine, and fecal samples was analyzed after wet digestion by the method as described by Fenton and Fenton (1979), using nitric acid and 70% perchloric acid. Chromium concentration was determined by measuring absorption using a spectrophotometer at 450 nm (Spectronic 21D, Milton Roy Co., Rochester, NY). The concentration of P was analyzed by measuring absorption using a spectrophotometer at 620 nm (Spectronic 21D, Milton Roy

Co., Rochester, NY) after adding Fiske-Subbarow reducer solution and acid molybdate to digested samples to develop a blue color.

6.3.4 Calculation and Statistical Analysis

The ATTD (%) and TTTD (%) of nutrients were calculated by equations described by Zhai and Adeola (2013):

$$ATTD = 100 \times (Nutrient_I - Nutrient_O) / Nutrient_I,$$

$$P_D = (C_{TTTD} \times P_I) + Intercept,$$

$$TTTD = C_{TTTD} \times 100,$$

Where the $Nutrient_I$ is the dietary N or P intake (g/d); the $Nutrient_O$ is fecal N or P output (g/d); P_D is digested P (g/d); C_{TTTD} is the coefficient of TTTD estimated by regressing P_D against P_I within each of the three CP concentrations using GLM procedure of SAS 9.4 (SAS Institute, 2006). The ATTR of N or P was derived by subtracting urine N or P output (g/d) from the digested N of P (g/d). The difference between slopes (low vs. medium dietary CP; low vs. high dietary CP; and medium vs. high dietary CP) was analyzed as described by Xue and Adeola (2015), using the GLM procedure of SAS. The statistical model is:

$$P_D = Level_{CP} \quad P_I \quad Level_{CP} * P_I,$$

where P_D is digested P (g/d); $Level_{CP}$ was coded as a dummy variable (0 or 1, for two of the three dietary CP concentrations) and considered as class variable; P_I is dietary P intake (g/d) and was not included in the class statement; and $Level_{CP}$ $*P_I$ is the interaction of $Level_{CP}$ and P_I.

The digestion and retention data for N and P were analyzed using the MIXED procedure of SAS 9.4 (SAS Institute, 2006). The dietary N and P concentrations were considered as fixed effect and the block of BW was considered as random effect. Individual pig was the experimental unit for all statistical analyses. Statistical significance was determined at $P < 0.05$. The REG procedure of SAS (9.4) (SAS Institute, 2006) was used to determine the quantitative relationship between apparent total tract N and P retention (g/pig). In the regression analysis, the apparent total tract retention of P (g/pig) was regressed against the apparent total tract retention of N (g/pig).

6.4 Results

The analyzed composition of nutrients in experimental diets were close to the calculated values. There was a dietary CP effect on the average BW gain (kg/pig) over the 10-d experimental period ($P < 0.001$). The average BW gain for the low, medium, and high CP groups were 0.76, 2.02, and 2.47 kg/pig, respectively. The effects of ATTDP concentration and the interaction between dietary CP and ATTDP concentrations were not observed.

The results of N digestion and retention in response to dietary CP and ATTDP concentrations are presented in Table 6-2. The fecal output (g/d), urine output (g/d), ATTD, and ATTR of N was increased linearly along with the increasing dietary CP concentration ($P < 0.001$). The digestion and retention of N increased in both linear and quadratic patterns with the increasing concentration of dietary CP ($P < 0.001$). The dietary ATTDP concentration affected the urine N output and there was an interaction between CP and ATTDP concentration in the urine N output ($P < 0.05$). The urine N output linearly decreased with the ATTDP concentration in the low and medium CP concentrations, and also decreased in a quadratic pattern in the low CP group ($P < 0.05$).

The response of P digestion and retention to different dietary CP and ATTDP concentrations is in Table 6-3. The fecal output (g/d), digested (g/d), and retained (g/d) P was linearly increased by the increasing concentration of CP in the diets ($P < 0.05$). The response was similar to P concentrations, except for both linear and quadratic responses of P digestion and retention (g/d) to ATTDP concentrations observed in the medium CP group ($P < 0.05$). The ATTD and ATTR of P linearly increased by the addition of MCP in diets and decreased by the greater inclusion rate of corn and SBM in experimental diets.

The linear regression coefficients are shown in Table 6-4. The regression equation for low CP diets is Y = 0.805 (SE = 0.063) X – 2.867 (SE = 0.914), $R^2 = 0.93$. For medium CP diets, the regression equation is Y = 0.826 (SE = 0.054) X – 2.867 (SE = 0.929), $R^2 = 0.95$. The equation for high CP concentrations is Y = 0.879 (SE = 0.067) X – 7.029 (SE = 1.263), $R^2 = 0.94$. Therefore, the TTTD of P in MCP was 80.5, 82.6, and 87.9%, for low, medium, and high CP concentrations, respectively. There was no difference between the three slopes.

The polynomial regression of apparent total tract retention (g/pig) of P against apparent total tract retention of N (g/pig) for the 9 treatments is shown in Figure 6-1. The polynomial regression equation was Y = -0.0013 (SE = 0.001) X^2 + 0.1622 (SE = 0.112) X + 2.8706 (SE = 2.432), $R^2 = 0.0879$.

6.5 Discussion

Protein nutrition is very important to swine, because the digestion and retention of protein are directly linked to the accretion of tissues. For this reason, there are plenty of studies focused on the ileal digestibility of AA and the total tract retention of N, and the factors that may affect the nutrition of protein in pigs (Pahm et al., 2008; Eklund et al., 2015; He et al., 2016). Theoretically, the determined apparent digestibility of N, both ileal and total tract digestibilites, should be increased along with the dietary CP concentration (Fan and Sauer, 1995; Fan and Sauer, 1997). It can be explained by the relatively smaller portion of endogenous loss in the total CP or AA outflow, in animals fed with high CP diets (Stein et al., 2007). This phenomena has been observed in several previous studies (Fan and Sauer, 1997; Stein et al., 2005; Xue et al., 2014), as well as in the current experiment, where the ATTD and ATTR of N were linearly increased from the low to high dietary CP concentrations. For this reason, it is advocated that standardized ileal digestibility is a better measurement of AA digestibility, and the evaluation of AID should be performed with experimental diets that are formulated to contain a moderate concentration of protein (Adeola et al., 2016).

There is not a lot of study focused on the influence of dietary P concentration on CP and AA digestibility. In one previous study from our lab, it was determined that the dietary ATTDP concentration exerts little influence on the determination of AID and SID of AA (Xue et al., 2015). Similar results was observed in ATTD and ATTR of N in the current study, where ATTDP in diets did not impact the determination. However, there was an influence of dietary ATTDP concentrations and interaction between dietary CP and ATTDP concentrations on urine N output in this study. It was demonstrated in the current study that the increasing dietary ATTDP concentration linearly decreased the urine N output in the low and medium CP groups. The major component of N in urine is urea, which is the metabolite of protein metabolism in the animal body. It can be considered as an indicator of protein utilization (Kerr and Easter, 1995). This result indicates the dietary ATTDP content may impact the utilization of protein in growing pigs. But the ATTR of N was not affected by dietary ATTDP concentrations, due to the relatively smaller portion of urine N loss, compared with the amount of total tract digested N. This result was consistent with previous data reported by Adeola et al. (2015).

In the current study, the diets were formulated on the basis of ATTDP, rather than total P. The increasing concentration of corn and SBM in the three dietary CP concentrations led to different total P concentrations among the low, medium, and high ATTDP groups (Table 6-1). For this reason, the comparison between determined P digestibilities within each ATTDP concentration was biased. The discussion of P utilization will focus on digested P in g/d basis rather than the digestion and retention coefficients in the rest of the manuscript.

The dietary ATTDP concentrations were adjusted by the supplementation of MCP in the diets. The MCP was reported to have greater TTTD of P compared with corn or SBM (Zhai and Adeola, 2012; Zhai and Adeola, 2013). Thus, as we expected, the increasing concentration of MCP in diets linearly increased the ATTD and ATTR of P within each CP concentration. The main effect of dietary CP on P digestion and retention (g/d) was observed in this study. The digestion and retention of P was increased by the increasing dietary CP concentration. This result is consistent with the previous study from our lab on growing pigs and broiler chickens (Xue et al., 2015, 2016). In addition, it should be noted that the pattern of P digestion and retention response to dietary ATTDP concentration was different among the three dietary CP concentrations. The P digestion and retention increased in a quadratic pattern in the low CP group, while the response was linear in the medium and high CP groups. These results indicated that the dietary CP concentration may affect the total tract digestion and retention of P in growing pigs.

The determined TTTD of P in MCP was 80.5, 82.6, and 87.9% in low, medium, and high CP diet, respectively. Although the TTTD of P derived from the three dietary CP concentrations numerically increased from 80.5% to 87.9%, the slopes were not statistically different. This could be a result of the wide confidence interval of the slope in linear regression. This result indicated that the CP concentration in the basal diet did not affect the determination of TTTD of P in MCP by the regression method in the current study, which is in agreement with previous studies (Liu et al., 2014a, b). However, the TTTD of P in MCP increased approximately 9.2% when the dietary CP concentration increased from 5.5% to 13.9%. The numerical difference can still be considerable when the values were used to formulate diets for swine.

The regression method is applied to determine the TTTD of P in feed ingredients (Akinmusire and Adeola, 2009; Xue and Adeola, 2015). It was assumed that the basal diet composition may not impact the determination of nutrient and energy digestibilites determined by the regression method. But the physiological status of animals fed with semi-purified diets during the experimental period is questionable. To prevent this potential abnormal physiological status of the animal, protein supplementation such as casein was suggested to be added in the experimental diets (Adeola et al., 2016). A number of studies indicated that the there was no statistical difference between the estimates derived from different basal diets. Liu et al. (2014a) reported that casein supplementation in the basal diet did not affect the determination of TTTD of P in SBM for broiler chickens. Shastak et al. (2014) reported similar results that basal diet type (corn-SBM vs. semi-purified diet with potato protein; corn vs. wheat) did not affect the TTTD of P in monosodium phosphate for broiler chickens. Previous studies in pigs suggested a similar conclusion. It was reported by Liu et al. (2014b) that semi-purified or corn based diets did not affect the estimation of TTTD of P in SBM for growing pigs using the regression method. However, in those previous studies, the difference of dietary CP concentrations between basal diet types were not as dramatic as it was in the current study. The relatively larger numerical difference of TTTD derived from the varying dietary CP concentrations in the current study indicated that the determination of P digestibility using the regression method may be affected by severe protein deficiency. This limiting effect might not be observed in moderate low protein diets.

In another previous study, it was suggested that egg albumen supplementation in the basal diet decreased the TTTD of P determination in corn and SBM using the regression method in broiler chickens (Mutucumarana et al., 2015). This result was opposite to the data in the current study, where the TTTD of P was numerically greater in high CP diets. A plausible explanation can be that the total P concentration in the albumen added diets was much greater compared with the diets without albumen. The excessive P concentration in the small intestine decreased the efficiency of P digestion.

As indicated in the NRC (2012), the whole body composition of N and P in growing pigs are correlated with each other. For these two aspects, it is reasonable to hypothesize that the deposition of protein and P may be quantitatively correlated. It was indicated in the previous study that the total tract retention of P was correlated with the total tract retention of N in both linear and quadratic pattern in broiler chickens (Xue et al., 2016). However, the correlation between the retention of N and P in growing pigs was not observed in the current study. Similar results were reported by Adeola et al. (Adeola et al., 2015), which indicated the total tract retention of N was not affected by the dietary P concentration. Although the quantitative relationship of N and P retention was not detected in these studies, it is too early to draw the conclusion that the relationship does not exist in growing pigs. The correlation between body composition of N and P reported by NRC (2012) was summarized based on data from a wide range of BW. Therefore, the correlation between the deposition of N and P, if it exists, should be also synchronized with BW gain of the animal. In the current and previous studies, the feed allowance of animals was limited to perform the total collection method, which led to a result that the BW gain was not different among the increasing dietary ATTDP concentrations (Adeola et al. 2015). On the contrary, in the previous study in broiler chickens, in which the correlation between N and P retention was observed, there was a linear increase in BW gain along with the increasing ATTDP concentrations in the low CP diets (Xue et al., 2016). Thus, this phenomenon suggested that BW gain is the driving factor for this quantitative relationship. Future study is needed to investigate this relationship with an animal model that has a wide range of BW gain.

In conclusion, the results indicated that dietary CP deficiency may limit total tract P digestion and retention. The dietary P deficiency may also impact the protein metabolism in growing pigs. Further studies with animal models that have a wide range of BW gain are still needed to investigate the quantitative relationship between N and P retention and its mechanism in growing pigs.

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Table. 6-1 Diet formulation and nutrient composition of diets.

CP level[1], %	5.6			9.7			13.9		
Apparent total tract digestible P level[2],%	0.11	0.19	0.27	0.11	0.19	0.27	0.11	0.19	0.27
Corn	188.0	188.0	188.0	329.0	329.0	329.0	470.0	470.0	470.0
SBM	80.0	80.0	80.0	140.0	140.0	140.0	200.0	200.0	200.0
Cornstarch	663.5	656.3	649.3	487.8	480.6	473.5	304.1	296.9	289.8
Soy oil	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Monocalcium phosphate	3.4	7.4	11.3	2.1	6.1	10.0	0.8	4.8	8.7
Limestone	4.1	7.3	10.4	4.1	7.3	10.5	4.1	7.3	10.5
Solka-floc	40.0	40.0	40.0	16.0	16.0	16.0	0.0	0.0	0.0
Salt	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Vitamin premix[3]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Mineral premix[4]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Selenium premix[5]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000
Calculated Nutrients & Energy									
Protein, g/kg	55.5	55.5	55.5	97.2	97.2	97.2	138.8	138.8	138.8
ME, kcal/kg	3797.4	3768.7	3740.8	3867.7	3839.0	3810.7	3906.1	3877.4	3849.2
Ca, g/kg	2.38	4.24	6.04	2.39	4.24	6.08	2.39	4.25	6.08
ATTD P, g/kg	1.08	1.92	2.74	1.08	1.92	2.74	1.08	1.92	2.74
Ca:ATTDP	2.21	2.21	2.21	2.22	2.21	2.22	2.22	2.22	2.22
Total P	1.83	2.67	3.49	2.39	3.23	4.05	2.96	3.80	4.62

[1]The 3 CP levels refers to low, medium, and high CP diets, respectively.

[2]The 3 ATTDP levels refers to low, medium, and high ATTDP diets, respectively.

[3]Vitamin premix supplied per kg of diet: vitamin A, 2,423 IU; vitamin D_3, 242 IU; vitamin E, 17.6 IU; vitamin K activity, 2.4 mg; menadione, 804 μg; vitamin B_{12}, 14.1 μg; riboflavin, 2.8 mg; d-pantothenic acid, 9 mg; and niacin, 13 mg.

[4]Mineral premix supplied per kg of diet: Cu (as copper sulfate), 9 mg; I (as calcium iodate), 0.34 mg; Fe (as ferrous sulfate), 97 mg; Mn (as manganese oxide), 12 mg; and Zn (as zinc oxide), 97 mg.

[5]Supplied 300 μg of Se per kg of diet.

Table 6-2. Response of total tract CP digestion and retention to dietary CP and ATTDP[1] concentrations.

Items	Nitrogen intake, g/d	Fecal Nitrogen output, g/d	Urine Nitrogen output, g/d	Digested Nitrogen, g/d	Retained Nitrogen, g/d	ATTD[2] of N, %	ATTR[3] of N, %
Low CP diets, 5.5% CP							
0.11% ATTDP[4]	8.62	1.82	1.96	6.80	4.84	78.52	55.53
0.19% ATTDP	8.51	2.00	1.68	6.56	4.95	76.34	57.22
0.27% ATTDP	8.52	1.96	1.60	6.57	4.97	76.80	57.93
Medium CP diets, 9.7% CP							
0.11% ATTDP	13.39	2.31	3.48	11.08	7.67	82.77	57.02
0.19% ATTDP	13.39	2.51	2.74	10.88	8.05	81.26	60.04
0.27% ATTDP	13.39	2.52	2.93	10.87	7.94	81.25	59.18
High CP diets, 13.9% CP							
0.11% ATTDP	20.87	2.66	4.13	18.21	14.08	87.23	67.03
0.19% ATTDP	20.81	3.00	4.34	17.81	13.47	85.60	64.59
0.27% ATTDP	20.81	3.11	4.00	17.70	13.60	85.12	65.61
SEM	0.15	0.15	0.13	0.18	0.23	1.15	1.60
P-value for main effects and interaction							
CP level	-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P level	-	0.071	0.004	0.070	0.976	0.103	0.697
CP level × P level	-	0.872	0.017	0.919	0.230	0.995	0.450
P-value for contrasts of CP level							
Low P linear CP	-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Low P quadratic CP	-	0.697	0.009	<0.001	<0.001	0.939	0.032
Medium P linear CP	-	<0.001	<0.001	<0.001	<0.001	<0.001	0.001
Medium P quadratic CP	-	0.936	0.098	<0.001	<0.001	0.842	0.656
High P linear CP	-	<0.001	<0.001	<0.001	<0.001	<0.001	0.001
High P quadratic CP	-	0.936	0.395	<0.001	<0.001	0.839	0.164
P-value for contrasts of P level							
Low CP linear P	-	0.532	0.040	0.355	0.659	0.296	0.257
Low CP quadratic P	-	0.565	0.507	0.598	0.875	0.379	0.798
Medium CP linear P	-	0.330	0.004	0.414	0.388	0.354	0.327
Medium CP quadratic P	-	0.593	0.006	0.651	0.378	0.597	0.320
High CP linear P	-	0.042	0.493	0.051	0.140	0.199	0.531
High CP quadratic P	-	0.543	0.081	0.511	0.174	0.683	0.358

[1]Apparent total tract digestible P.

[2]Apparent total tract digestibility.

[3]Apparent total tract retention.

[4]The 3 ATTDP levels refer to low, medium, and high ATTDP diets, respectively.

Table 6-3. Response of total tract P digestion and retention to dietary CP and ATTDP[1] concentrations.

Item	P intake, g/d	Fecal P output, g/d	Urine P output, g/d	Digested P, g/d	Retained P, g/d	ATTD[2] of P, %	ATTR[3] of P, %
Low CP diets, 5.5% CP							
0.11% ATTDP[4]	1.50	0.84	0.01	0.66	0.64	43.47	42.42
0.19% ATTDP	2.26	1.10	0.01	1.17	1.15	51.89	51.29
0.27% ATTDP	3.03	1.12	0.01	1.90	1.89	62.85	62.53
Medium CP diets, 9.7% CP							
0.11% ATTDP	2.14	1.16	0.02	0.98	0.96	45.63	44.82
0.19% ATTDP	2.76	1.37	0.01	1.39	1.38	50.34	49.92
0.27% ATTDP	3.62	1.53	0.01	2.17	2.16	60.12	59.85
High CP diets, 13.9% CP							
0.11% ATTDP	2.50	1.53	0.03	0.91	0.88	35.78	34.64
0.19% ATTDP	3.34	1.77	0.01	1.57	1.58	46.81	46.42
0.27% ATTDP	4.05	1.80	0.02	2.25	2.23	55.84	55.49
SEM	0.03	0.07	0.02	0.07	0.06	2.43	2.48
P-value for main effects and interaction							
CP level	-	<0.001	0.263	<0.001	<0.001	0.012	0.009
P level	-	<0.001	0.203	<0.001	<0.001	<0.001	<0.001
CP level × P level	-	0.856	0.438	0.453	0.343	0.610	0.913
P-value for contrasts of CP level							
Low P linear CP	-	<0.001	0.651	0.009	0.014	0.036	0.038
Low P quadratic CP	-	0.748	0.631	0.018	0.015	0.052	0.047
Medium P linear CP	-	<0.001	0.275	<0.001	<0.001	0.146	0.172
Medium P quadratic CP	-	0.427	0.833	0.783	0.783	0.899	0.741
High P linear CP	-	<0.001	0.326	0.001	0.001	0.066	0.068
High P quadratic CP	-	0.441	0.040	0.261	0.258	0.806	0.795
P-value for contrasts of P level							
Low CP linear P	-	0.004	0.066	<0.001	<0.001	<0.001	<0.001
Low CP quadratic P	-	0.155	0.725	0.151	0.169	0.678	0.698
Medium CP linear P	-	<0.001	0.536	<0.001	<0.001	<0.001	<0.001
Medium CP quadratic P	-	0.726	0.940	0.023	0.023	0.026	0.406
High CP linear P	-	0.012	0.158	<0.001	<0.001	<0.001	<0.001
High CP quadratic P	-	0.216	0.436	0.904	0.778	0.743	0.667

[1]Apparent total tract digestible P.

[2]Apparent total tract digestibility.

[3]Apparent total tract retention.

[4]The 3 ATTDP levels refer to low, medium, and high ATTDP diets, respectively.

Table 6-4. Linear regression coefficients and estimation of TTTD of P in mono-calcium phosphate[1].

Item	Intercept	SE of intercept	Slope	SE of slope	Estimated TTTD of P, %	R^2
Low CP diets, 5.5% CP	-2.867	0.914	0.805	0.063	80.5	0.93
Medium CP diets, 9.7% CP	-3.342	0.929	0.826	0.054	82.6	0.95
High CP diets, 13.9% CP	-7.029	1.263	0.879	0.067	87.9	0.94

[1]TTTD = true total tract digestibility; total tract digested P (g/kg DMI) in low, med, and high dietary ATTDP diets was regressed against dietary P intake (g/kg DMI) in each of the three protein concentrations. The statistical model was P_D = TTTD * P_I + intercept, where P_D is ileal digested P (g/kg DMI); P_I is dietary P intake (g/kg DMI). The regression slope represented an estimation of true ileal P digestibility of mono-calcium phosphate. The slopes were not statistically different from each other.

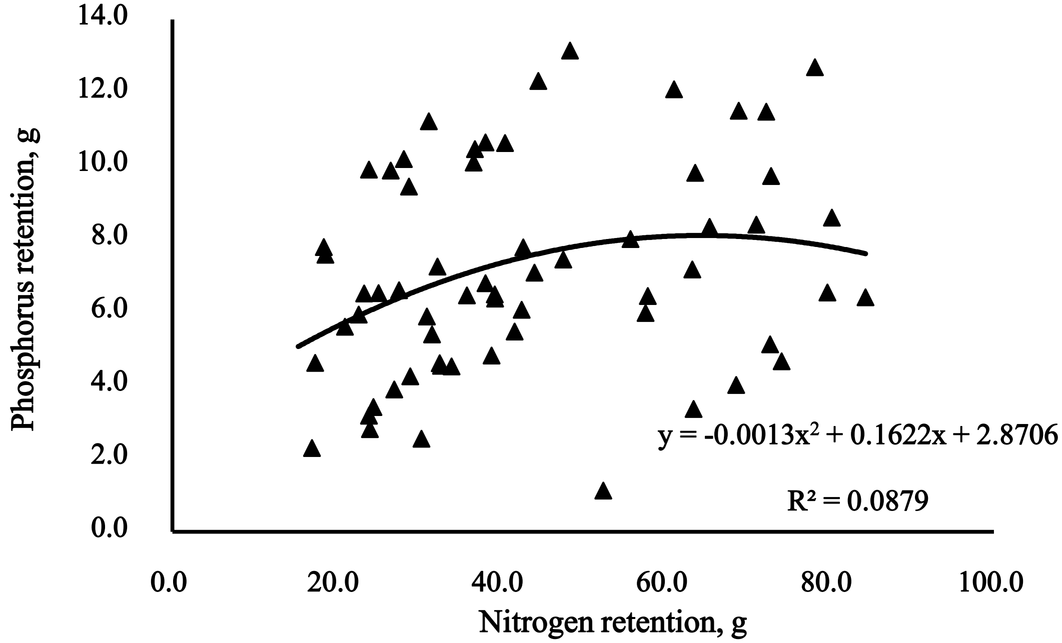

Figure 6-1. Relationship between apparent total tract N and P retention (g/pig). Each data point represents a pig. Apparent total tract retention of P (g/pig) was regressed against apparent total tract retention of N (g/pig). The polynomial regression equation was Y = -0.0013 (SE = 0.001, P = 0.270) X^2 + 0.1622 (SE = 0.112, P = 0.154) X + 2.8706 (SE = 2.432, P = 0.243), R^2 = 0.088.

CHAPTER 7. SUMMARY

7.1 Summary

For the optimization of dietary formulation, it is important to determine the digestibility and retention of nutrients, such as AA and minerals, in feed ingredients for growing pigs and broiler chickens. In the previous studies working on this topic, the utilization of nutrients was investigated separately. In this research, we investigated the methodology for the determination of two macro-nutrients, protein and P, and the relationship between the digestion and retention of these two aspects in growing pigs and broiler chickens.

In chapter 1, the methodology of the determination of AA and P digestibilites and the endogenous losses of AA and P in growing pigs and broiler chickens is reviewed. The nitrogen-free diet is recommended to be included in the studies that focused on ileal digestibility of AA to estimate BEL and SID of AA. The regression method can be used to determine the TID or TTTD (TTTR for poultry) of P in feed ingredients, with the dietary P concentrations below a marginal deficiency. However, the regression method is not preferred to estimate the endogenous P losses (EPL), due to the relatively wider confidence interval when the linear regression is extrapolated to zero. In addition, the regression method cannot be used to determine EPL if the basal diet contains a P source other than the candidate ingredient.

Chapter 2 included a study conducted in growing pigs to investigate the additivity of AID or SID of CP and AA in mixed diets containing multiple protein sources. Eighteen growing pigs (initial BW = 61.3 ± 5.5 kg) with surgically fitted T-cannula were assigned to a duplicated 9 × 4 incomplete Latin square design with 9 diets and 4 periods. A nitrogen-free diet was included to estimate BEL of AA. The AID and SID of AA in corn, SBM, DDGS, and canola meal was determined. The predicted and determined AID and SID of AA in mixed diets (corn-SBM, corn-SBM-DDGS, corn-SBM-canola meal, and corn-SBM-DDGS-canola meal) were compared. The results substantiate the notion that SID of AA are more accurate than AID for predicting ileal digestibility of AA in mixed diets containing multiple protein sources.

Chapter 3 included the application of the regression method in the determination of TTTD of P. A total of 48 barrows (initial BW 22.2 ± 1.3 kg) were assigned to the 6 diets in a randomized complete block design, to determine the TTTD of P in triticale DDGS for growing pigs with or without phytase. The six diets were formulated in a 3 × 2 factorial arrangement, including 3 concentrations of triticale DDGS (300, 400, or 500 g/kg) and 2 concentrations of phytase (0 or 500 FTU/kg of diet). The TTTD of P was determined to be 75.4% for triticale DDGS or 81.1% with added phytase, respectively. Although the difference was not statistically significant, the addition of phytase improved ATTD of P in triticale DDGS. In diets without added phytase, the ATTD of P in triticale DDGS was 65.0, 67.7, or 63.2% for the diets with 300, 400, or 500 g/kg triticale DDGS, respectively; the corresponding values for diets with added phytase were 77.3, 76.3, or 75.7%. For triticale DDGS, the supplementation of 500 FTU/kg phytase in diet could increase the ATTD of P ($P < 0.001$), but not the TTTD of P.

In chapter 4, an experiment was conducted to investigate the effect of dietary CP concentrations on ileal P digestion in growing pigs. The true ileal digestibility (TID) of P in mono-calcium phosphate (MCP) was determined in three CP concentrations. A total of 18 ileal-cannulated pigs (initial BW 44.2 ± 3.2 kg) were used in a duplicated 9 × 3 incomplete Latin Square design, with 9 treatments and three 7-d experimental periods giving 6 replicates per treatment. The 9 treatments consisted of one nitrogen-free diet to estimate BEL of AA, and 8 corn-soybean meal-based diets in a 2 × 4 factorial arrangement, which included 2 CP concentrations (6.9 or 13.4%) and 4 apparent total tract digestible P (ATTDP) concentrations (0.09, 0.16, 0.24, or 0.32%). In the results, low CP diets limited ileal digested P (g/kg•DMI). The ileal digested P (g/kg•DMI) increased linearly with increasing ATTDP concentrations in low CP group, but the pattern was linear and quadratic in the high CP group. In low and high CP diets, the determined TID of P in MCP was 54.4% and 75.6%, respectively. This study indicated that the ileal digestion of P could be limited by protein deficiency. The dietary CP concentration should be considered in P digestibility related studies.

The quantitative relationship between N and P digestion and retention in broiler chickens was determined in chapter 5. A total of 384 14-d-old male broiler chickens were used in a randomized complete block design with 8 treatments and 6 replicates per treatment in a 7-d experimental period. There were 8 corn-soybean meal-based diets in a 2 × 4 factorial arrangement, which included 2 CP concentrations (10.7 or 21.5%) and 4 ATTDP concentrations (0.18, 0.32, 0.45, or 0.59%). Low dietary CP concentration limited the growth performance, pre-cecal digestion and total tract retention of P, and NaPi-IIb gene expression. The total tract retention of N and P was correlated following

2nd order polynomial regression equation: Y = -0.012 (SE=0.005, $P < 0.05$) X^2 + 0.752 (SE=0.094, $P < 0.01$) X – 1.279 (SE=0.365, P=0.69), (R^2=0.618), where X is the total tract retention of N (g), and Y is the total tract retention of P (g). This study suggests an interrelationship between N and P digestion and retention such that CP deficiency decreased the growth performance of birds consequently reducing P digestion and retention in broiler chickens.

Chapter 6 included a study that investigated the quantitative relationship between N and P retention in growing pigs. A total of 72 growing pigs (initial BW 20.9 ± 0.8 kg) were used in a randomized complete block design, with 9 treatments and four 10-d experimental periods giving 8 replicates per treatment. The pigs were blocked by BW and allotted to 9 treatments with a 3 × 3 factorial arrangement consisting 3 CP concentrations (5.5, 9.7, or 13.9%) and 3 ATTDP concentrations (0.11, 0.19, or 0.27%). In the results, the determined TTTD of P in MCP for 5.5, 9.7, and 13.9% CP diets were 80.5, 82.6, and increasing dietary P concentration decreased the urine nitrogen output. Yet the quantitative relationship between total tract N and P retention was not observed in this study.

In previous studies, it was reported that the apparent ileal digestibility (AID) of AA in feed ingredients is not additive in the complete diet, because the endogenous loss of AA in the ileal AA flow is not considered (Stein et al., 2005). Due to the adjustment of the basal endogenous loss (BEL) of AA, the standardized ileal digestibility of AA is assumed to be additive in the complete diet (Stein et al., 2007). Thus, it is important to validate the additivity assumption of SID of AA in the complete diet. In chapter 2 we demonstrated that the SID of most AA in corn, SBM, corn DDGS, and canola meal are additive in mixed diets using those ingredients.

The regression method can be used to determine the true ileal (TID) and total tract (TTTD) digestibility of P in feed ingredients, as well as the endogenous P losses (EPL) at the same time (Fan et al., 2001; Dilger and Adeola, 2006). In chapter 3, we applied the regression method to determine the TTTD of P in triticale DDGS. The results indicated that the regression method can be used to determine the TTTD of P. However, the estimation of EPL in the regression method is not recommended due to the high variation resulting from the extrapolation.

It is summarized in the NRC (2012) that the whole body composition of N and P in growing pigs are correlated in a 2nd order polynomial model. Based on this correlation between N and P composition, the requirement of P for growing pigs in the NRC (2012) is estimated by using the protein accretion model (NRC, 2012). In fact, the results of P requirement for growing pigs from empirical studies agrees with the estimations in the NRC (2012), which is generated by the factorial method. (Zhai and Adeola, 2013a, b). This agreement indicates that the accretion of protein is also correlated to the deposition of P. Thus, it is reasonable to assume that the deposition of N and P are also correlated with each other during BW growth. To investigate the relationship between N and P deposition in growing pigs and broiler chickens, we determined the interaction between dietary N and P from ileal digestion level to total tract retention level. The results indicated that the determination of digestion of P might be affected by dietary CP concentrations. This phenomenon suggested that CP deficiency might impact the digestion and absorption of P in feed ingredients. This limiting effect might partly be explained by the down regulation of sodium-dependent phosphate transporter II-b (NaPiII-b) gene expression in CP-deficient diets. In the total tract retention level, the deposition of N and P are correlated with each other in a quadratic pattern in broiler chickens. Yet this correlation was not observed in growing pigs. A plausible explanation of this phenomenon can be the difference in BW gain between the two studies. The experimental period in the growing pig study was relatively shorter, and the feed intake was limited to perform the total collection of feces. For this reason, there was no effect on P concentrations to BW gain, whereas the difference was observed in the broiler chicken study. Therefore, these results indicate that BW gain might be the driving factor for the correlation between N and P retention.

Further study is needed to investigate the quantitative relationship between total tract N and P retention in growing pigs and broiler chickens. It was demonstrated in chapter 5 that in broiler chickens, the relationship between N and P retention is approximately 10:1, which is similar to the average value reported by some previous studies. This quantitative relationship need to be illustrated and confirmed in further studies. Future studies in growing pigs are still needed to investigate this relationship in an animal model with a wide BW gain range. The feed intake level should be maintained close to ad libitum to reveal the correlation between N and P retention in a normal physiological scenario.

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Nutritional evaluation of enzymes and other additives.

PUBLICATIONS

PUBLICATIONS

Xue, P. C., K.M. Ajuwon, and O. Adeola. 2016. Influence of dietary crude protein levels on phosphorus ileal digestion, total tract retention, and small intestine P transporter gene expression in broiler chicken. Poult. Sci. 95(11): 2615-2623.

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